# COMPARISON OF RABBIT POLYCLONAL ANTI-IDIOTYPES TO BOVINE HERPESVIRUS-1

by

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# REVIEW OF LITERATURE

# LITERATURE REVIEW

## PROPERTIES OF THE VIRUS

Bovine herpesvirus 1 (BHV-1), also known as IBR virus and IPV virus, is responsible for a complex of clinical manifestations in cattle , some of which include: rhinitis, tracheitis, vulvovaginitis, abortion, conjunctivitis, and encephalitis (Gibbs et al, 1977). BHV-1 is a member of the family Herpesviridae; subfamily alphaherpesvirinae (Matthews, 1982; Roizman and Batterson, 1985). It is a DNA virus as was first suggested by Armstrong et al (1961), double-stranded with an average molecular mass of $84x10^6$ daltons (Seal et al, 1985). BHV-1 particles have a diameter of 145 to 156 nm (Tousimis et al, 1958). The capsid contains 162 hollow capsomeres arranged in an icosahedral symmetry; an envelope surrounds the nucleocapsid derived from the nuclear membrane (Watrach and Bahneman, 1966; Bocciarelli et al, 1966). BHV-1 specifies 33 structural polypeptides, 11 of which are glycosylated (Misra et al, 1981; Bolton et al, 1983; Chang et al, 1986). Ten glycoproteins with molecular masses ranging from 45Kd to 180Kd (Marshall et al, 1986), and one non-glycosylted 107Kd protein (Chang et al, 1986; Marshall et al, 1986) were located on the viral surface. Immunoprecipitations with monospecific and monoclonal antibodies showed 3 sets of coprecipitating glycoproteins, 180Kd/97Kd, 150Kd/77Kd, and 130Kd/74Kd/55Kd, were the major components of the BHV-1 envelope and were also present on the surface of BHV-1-

infected cells (Marshall et al, 1986). 180Kd/97Kd and 150Kd/77Kd glycoproteins contain epitopes which induce neutralizing antibodies (Van Drunen Littel-van den Hurk and Babiuk, 1985; Marshall et al, 1986). Other glycoproteins or glycoprotein complexes were identified by investigators (Bolton et al, 1983; Van Drunen Littel-van den Hurk and Babiuk, 1985; Collins et al, 1985a). The glycoproteins on the viral envelope are pivotal in the virus-host relationship since they are involved in the recognition, attachment, and penetration of herpesviruses into susceptible cells (Little et al, 1981), viral neutralization (Glorioso et al, 1984; Lupton et al, 1980) and in the immune destruction of infected cells (Bishop et al, 1981; Carter et al, 1981; Rouse et al, 1984)

The various strains of IBR virus are antigenically homogeneous, although slight differences between them have been demonstrated by neutralization tests in tissue culture (York et al, 1957). Seal et al (1985) demonstrated differences between genomes of several viral isolates of BHV-1 from different forms of the disease by DNA restriction enzyme analyses , but nucleic acid hybridization studies of viral DNAs indicated at least a 95% genetic homology. Studies performed with a number of BHV-1 isolates grouped them into 3 antigenic types on the basis of restriction endonuclease and protein analyses (Metzler et al, 1986; Friedli et al, 1987). The third group seems to differ biologically from the two other types as it shows neurotropic properties in calves (Bagust et al, 1972; Hall et al, 1966; Carrillo et al, 1983; Schudel et al, 1986). The 3 types can not be discriminated clearly by conventional serological methods (Metzler et al, 1986). Relationship of BHV-1 with equine rhinopneumonitis has been demonstrated by complement-fixation and gel diffusion (Carmichael and Barnes, 1961). IBR, Marek's and Burkitt's lymphoma viruses share a common antigen as shown by gel diffusion and indirect flourescence antibody techniques (Evans et al, 1972). BHV-1 and caprine herpesvirus-1 also have a common antigen (Berrios and McKercher, 1975). BHV-1 genome exhibits approximately 8% homology with pseudorabies virus genome (Bush and Prichett, 1985).

## CLINICAL MANIFESTATIONS ASSOCIATED WITH IBR VIRUS

Infectious bovine rhinotracheitis (IBR) has been described as "an acute, contagious febrile infection of cattle, characterized by an intense inflammation of the upper respiratory passages and trachea and accompanied by dyspnea, depression, nasal discharge, and loss of conditions" (McKercher et al, 1959). This form which was referred to as the respiratory form, was first recognized in beef cattle in Colorado feed lots (Madin et al, 1956). The genital form which is recognized as infectious pustular vulvovaginitis (IPV) occurs in heifers, dairy cows, and bulls (Kendrick et al, 1958) and is characterized by hyperemia of genital mucous membranes leading to pustule formation and ulceration (Smith et al, 1976). On occasion the major disease manifestation of the virus in a herd is meningoencephalitis in neonatal and young calves (French, 1962) or viremic disease in the same age group, with lesions in the upper portion of the digestive tract and in the parenchymatous organs (Baker et al, 1960); keratoconjunctivitis (Albinanti and Plumer, 1961); dermal infections; or abortions in cows (Chow et al, 1964; McKercher and Wada, 1964). BHV-1 has also been isolated from vesicular lesions of the bovine udder (Guy et al, 1984). Although cattle are believed to be the only significant natural host of the virus (McKercher, 1973), serological and isolation techniques have been used to implicate several other species in natural infections, including goats (Mohanty et al, 1972), pigs (Saxegaard and Onstad, 1967; Derbyshire and Caplan, 1976), and various widelife and zoological species (Stauber et al, 1980; Zarnke and Yuill, 1981).

## LATENT INFECTION

Latency, a biological property of great epidemiological importance, is shared by the herpesviridae (Honess and Watson, 1977). Although latency of BHV-1 in cattle is well documented (Pastoret et al, 1982; Homan and Easterday, 1981), the mechanisms of this process are still uncertain (Splitter et al, 1985). Models developed by studies with Herpes Simlex virus (HSV), probably apply to BHV-1 as well. A documented theory of HSV latency suggests that the virus travels up the trigeminal nerve and lies dormant after initial infection until a stimulus to the ganglion causes virus release (Splitter et al, 1985). Narita et al (1981) provided evidence that the trigeminal ganglion maybe a latent site for BHV-1. Moreover the isolation of IBR virus from the trigeminal ganglia of normal animals (Homan and Easterday, 1981) further supported the model mentioned earlier. Recently Rock et al (1986, 1987) were able to identify and map BHV-1 RNA in latently infected ganglionic tissues, using rabbit model with insitu nucleic acid hybridization.

Attentuated vaccine strains can remain in a latent state (Pastoret et al, 1980), even after usual vaccination procedure (Nettleton et al, 1984). Reactivation of BHV-1 can occur by means of several endogenous or exogenous stimuli, including the injection of glucocorticoids especially dexamethasone (Snowdon, 1965; Sheffy and Davis, 1972). The reactivation of latent virus may be followed by re-execretion of infectious particles (Snowdon, 1965), and non-infectious particles (Pastoret et al, 1979). The re-execretion of latent BHV-1 is modulated by the level of specific immunity, and whether this immunity was induced by the multiplication of a wild-type or attenuated virus (Straub and Wanger, 1977; Rossi and Kiesel, 1982).

## IMMUNITY TO INFECTION

The immune response of cattle to BHV-1 can be attributed to antibody and cell-mediated components (Gibbs and Rweyemanu, 1977; Wilkie, 1982), however upon initial herpesvirus infection, natural defense mechanisms play a role to limit and curtail the infection and may also have indirect effects on the recrudescent disease and extent of latency (Rouse, 1985). Adaptive immunity, which takes sometime to develope, assumes more importance in facilitating recovery and enhancing resistance to reinfection (Rouse, 1984a, b). Although humoral immune mechanisms play a significant role in reducing infection and limiting the spread of the virus (Babiuk et al, 1975), cell-mediated immunity (CMI) is believed to have a principal role in resolving infection (Rouse, 1985). Antibody-dependent cell-mediated cytoxicity (ADCC) has been demonstrated in vitro against BHV-1-infected cells and it occurs about the same time of intracellular transmission of the virus (Rouse et al, 1976). Complement-associated antibody-dependent cell-mediated cytoxicity (ADCC-C) utilizes complement components possibly C3b, which may bind to antibody and to the effector cell via a C3b receptor. Killing of virally infected cells by ADCC-C is more effective than ADCC alone (Rouse et al, 1977). IgM antibody functions in ADCC-C but not in ADCC suggesting that this phenomenon may play a role early in in vivo defense. Maternal antibodies transferred to the newborn in colostrum protected them against the respiratory form (Pospisil et al, 1983), and the fatal multisystemic form of IBR (Mechor et al, 1987). Maternal neutralizing antibodies are detected in serum of calves from immune dams, though titers are low, sometimes persist until the calf is 4 months old. Local humoral immune resonse at mucosal surfaces is mediated principally by dimeric locally synthesized, and scretory IgA (Strokes, 1985)

Humoral immune response also serves as an epidemiological tool. An animal with measurable neutralizing antibodies against 100 tissue culture infective doses ($TCID_{50}$) is immune. Titers occasionally reach 1:256, with the average ranging between 1:8 and 1:64 . The constant serum varying virus neutralization test (House and Baker, 1971) is reasonably sensitive in the detection of antibody. Other techniques employed to detect serum antibody include passive hemoagglutination (Whitman and Hetrick, 1965; Kirby et al, 1974), gel diffusion techniques (Kaminjolo and Gicho, 1972), complement-fixation test (Wellemans and Lenmen, 1973) and indirect flourescent antibody technique (Assaf et al, 1975). Enzyme-linked immunosorbent assay (ELISA) is an important tool with many advantages (Bommeli and Kihm, 1980, 1982). It has been shown to be more sensitive method for detecting antibody than serum neutralization test. Most recent studies involve the improvement of micro and macro ELISA as a rapid method of viral antigen detection and also for detection of antibody (Collins et al, 1985a, b, c, 1986, 1988; Darcel and Kozeib, 1984; Florent and de Marneffe, 1986; Herbert et al, 1985).

In studies of both immunodeffecient humans and immunosuppressed animals, the capacity to generate an antibody response can be normal in the face of sever herpesvirus infection (Meyers et al, 1980; Lopez et al, 1974). In such individuals , CMI is often suppressed. Such observations, along with the knowledge that herpesviruses can dessiminate in tissue culture in the presence of neutralizing antibody, are arguments commonly taken in support of a principal role for CMI in resolving infection. In vitro evidence of cytotoxic T lymphocytes (CTL) has been found in HSV. Bovine peripheral blood lymphocytes from BHV-1 immunized cattle, expressed cytotoxicity to BHV-1-infected cells. The cytotoxicity was found to be directly mediated by T cells and not attributable to ADCC (Rouse and Babiuk, 1977; Campos and Rossi, 1985). To show the role of CMI Rouse and Babiuk (1975) developed a viral plaque inhibition assay which demonstrated that blood lymphocyts from immune animals prevented viral plaque formation in cell monolayers infected with BHV-1. The reaction is specific and involves suppression of viral replication rather than the destruction of virus or virus-infected cells. The process is believed to be T cell mediated, and a lymphokine produced by the cooperation of macrophages and T lymphocytes is involved. Polymorphonuclear neutrophils in the presence of complement are also involved in CMI. The mechanism is described as complement-dependent neutrophil-mediated cytoxicity (CDNC), and its target is BHV-1-infected cells (Grewal et al, 1980; Grewal and Rouse, 1980).

Several studies have shown that BHV-1 infection in vivo produces high levels of interferon in blood (Rosenquist and

Loan, 1969) and nasal secretions (McLaughlin and Rosenquist, 1982; Cummins and Rosenquist, 1982). Interferon levels stimulated in cultures by BHV-1 antigen inhibited BHV-1 cell infection. Supernatants of virus-infected bovine macrophages possessed interfering activity (Forman et al, 1982). These findings suggessted that local production of interferon may play a role in control of virus dissemination in vivo (Spliter et al, 1985). Recent studies described levels of interferon in serum, and in the toxicity of E. coli-derived bovine alpha-2 interferon in dairy calves after intranasal, intramuscular, subcutaneous, and intravenous administration of interferon (Gillespie et al, 1986). In vitro protective effect of E. coli-derived bovine alpha-1 interferon against various strains of BHV-1 was of low order (Babiuk et al, 1985; Gillespie et al, 1985), nevertheless it ameliorated the resulting disease when compared to the controls, with lesser signs of illness and a delayed incubation period (Babiuk et al, 1985)

## VACCINATION

Immunity to viral infections depends on the development of an immune response to antigens present on the surface of virions and virus-infected cells. Available vaccines consist of attenuated live viruses, inactivated viruses, and subviral antigens. Inactivated virus vaccines prepared from whole virions, generally stimulate the development of circulating antibody, conferring some degree of resistance, but there may be some disadvantages associated with them: [1] Immunity conferred is often brief and must be boosted; [2] hypersensitivity development upon subsequent infection by wild virus; [3] perenteral administration produce little or no IgA response; [4] extreme care is required in their preparation to make certain that no live virulent virus remains in the vaccine. On the other hand live attenuated virus vaccines have the advantage of behaving like the natural infection in their effect on immunity, and generally stimulate longer-lasting antibody production, however they may have some disadvantages which include, the risk of reversion to virulence, dissemination of adventitious contaminants, and storage constraints. For many viral infections the application of live attenuated vaccines is contraindicated, or conventional vaccines are not available, or ineffective for a number of reasons, such as inability to grow the agent , unsatisfactory nature of attenuated strains, establishment of latency and shedding of the agent, therefore various approaches to the research and development of new vaccines for humans and animals are being actively explored.

The use of synthetic peptides as immunogens was first suggested by Langbeheim et al (1976). Rapid progress in determining RNA or DNA sequence of various viral genes has simplified the selection of peptides with potential antigenic activity (Murphy and Chanock, 1985). The feasibility of using synthetic peptides for inducing resistance in animals was demonstrated for foot-and-mouth disease virus (FMDV). An icosapeptide, corresponding to the major antigenic site of VP1 of FMDV, inoculated with Freund's adjuvant into ginea pigs protected them against challenge with wild-type FMDV (Bittle et al, 1982) Similar results were demonstrated with cattle (Bittle et al, 1984). Similar studies have been conducted with infleunza virus (Green et al, 1982; Arnon and Shapira, 1984), hepatitis B virus (HBV) (Gerin et al, 1984), and HSV (Dietzschold et al, 1984). With the development of DNA cloning techniques, RNA or DNA viral genes can be cloned in prokaryotic cells using a plasmid or bacteriophage vector. The cloned viral DNA can be expressed as viral proteins in bacteria. FMDV VP1 capsid protein (Kleid et al, 1981), HBV surface antigen (Edman et al, 1981), infleunza A virus hemagglutinin (Emtage et al, 1980), rabies virus glycoprotein (Yelverton et al, 1983), and HSV gD protein (Watson et al, 1982) have been expressed in bacteria.

The development of recombinant DNA technology and its use for genetic engineering has provided opportunities to construct, delete, or insert DNA sequences at specific sites of viral genomes (Moss, 1985; Arsenakis and Roizman, 1985). Live recombinant vaccinia virus vaccine is made by stable integration of one or more genes of important pathogens into the vaccinia virus genome. The constructed vaccinia virus hybrids express these genes efficiently, and maintain infectivity (Mackett et al, 1982; Panicali and Paoletti, 1982). The glycoprotein genes of the Indiana and NewJersey serotypes of vesicular stomatitis virus (VSV) were introduced into vaccinia virus (Mackett et al,1985). Intradermal inoculation of cattle with the recombinant vaccine conferred some degree of protection. The glycoprotein D gene of HSV has been inserted into vaccinia virus (Cremer et al, 1985; Paoletti et al, 1984). Mice inoculated with the recombinant vaccinia virus were protected against a lethal challenge with HSV-1 (Paoletti et al, 1984), or both HSV-1 and HSV-2 (Cremer et al, 1985), and against the development of latent HSV-1 infection. On the other hand the unique structure, the size, and the broad host range of the DNA genomes of HSV-1 and HSV-2, make them ideal vectors for the maintenance and expression of their own and of other organisms genes (Arsenakis and Roizman, 1985).

In the light of the knowledge of genetic basis of attenuation, newer techniques for construction of viruses with defined mutations or gene constellation are applied to the development of live virus vaccines. Attenuating mutations can be induced by chemical mutagenesis, deletion of a portion of the viral genome, or gene reassortment (Murphy and Chanock, 1985).

## ANTI-IDIOTYPES

Jerne's network theory (Jerne, 1974) implies that the immune system is maintained at an equilibrium by the interaction of complementary receptors expressed on immunocompetent lymphocytes. The variable region domains of these receptors (i.e, antibody molecules or T cell receptors) are thought to not only respond to external antigens, but also evoke an immune response in the autologous host. This concept, initially defined for antibody molecules, has been validated in experimental models (Oudin and Michel, 1963; Urbain et al, 1977), and has led investigators to propose that the immunogenicity of immunocompetent receptors might have practical applications for their use as vaccines to infectious agents (Nisonoff and Lamoyi, 1981).

Idiotypes (id) can be defined as unique epitopes within the variable regions of immunoglobulin (Ig) molecules produced by each individual B-cell clone, or within T-cell receptors on each individual T-cell clone (Lambert, 1986). Thus the anti-idiotypic (anti-id) arsenal comprises an anti-B cell receptor compartment, including anti-Ig id antibodies (Cosenza, 1976; Bona et al, 1978) and Ig id-specific T-cells (Bona and Paul, 1979; Bottomly and Mosier, 1979; Sherr et al, 1981, Jayarman and Bellone, 1982, Binz and Wigzell, 1978), and an anti-T-cell receptor compartment, including anti-T-receptor clonotypic antibodies (Staerz et al, 1985) and T-receptor-specific T-cells (Kimura and Wilson, 1984; Lancaster et al, 1985). Although T-cells have been integrated in the anti-id arsenal, the present knowledge of anti-id T-cells is rather limited (Lambert, 1986). Operationally, a part of the variable region domain of an antibody molecule, the paratope, binds to a complementary structure, the epitope, of the antigen. The paratope of the anti-id antibody binds to a complementary structure, the idiotope, of the antigen-binding antibody. Idiotopes within the paratope are called paratope-associated idiotopes, or paratactic idiotopes. Anti-id to paratactic idiotopes functionally mimic antigen, and can thus stimulate antigen-specific anti-id response. Such anti-id have been defined as Ab2ß antibodies, or "internal image" antibodies (Jerne et al, 1982). Part of the Ab2ß populations must have a three-dimentional structure which resembles an epitope of the antigen. Shared aminoacid sequences between the reference antigen and the variable region domain of the corresponding Ab2ß have been described in two systems employing protein antigens (Ollier et al, 1985; Bruck et al, 1986). The molecular basis of antigenic mimicry of polysaccharide determinants which has been described (Stein and Soderstrom, 1984), could possibly be explained by similarities in charge distribution (Finberg and Ertl, 1987). There are several examples which support the concept of internal image, demonstrating that anti-id can mimic various biologically active substances such as hormones (Sege and Paterson, 1978a), vitamins (Sege and Paterson, 1978b), viral (Kennedy and Dressman, 1984; Reagan et al, 1983; Uytdehaag and Osterhaus, 1985; Rubinstein et al, 1983), bacterial (Stein and Soderstrom, 1984; McNamara et al, 1985), and parastic antigens (Sachs, 1982; Grzyck et al, 1985). Other types of anti-id which include Ab2α (Jerne et al, 1982), Ab2ϵ (Bona, 1985), and autobody (Kang and Kohler, 1986) have been described.

Anti-id vaccines would have several advantages over conventional vaccines. They are less hazardous since they don't carry live organisms. Anti-id, by virtue of stimulating the immune system directly, may induce the production of antibodies not generated by the antigen itself (Francotte and Urbain, 1984), or even overcome unresponsiveness to the nominal antigen (Stein and Soderstrom, 1984). Some anti-id might be more effective in stimulating a T-cell response than inactivated vaccines (Finberg and Ertl, 1987).

The principle of idiotypic vaccination has been applied successfully for several viral systems (hiernaux, 1988). Kennedy et al (1984) have produced rabbit anti-id to hepatitis B surface antigen, which can induce virus- neutralizing antibody in mice. Recently, Kennedy et al (1986) reported the induction of protective antibody in Chimpanzee by anti-id. In the reovirus type 3 system, an anti-id monoclonal antibody raised against anti-reovirus type 3 monoclonal antibody, prevented the infectivity of the reovirus particles and their binding to target cells (Dichter et al, 1986); it triggered anti-reovirus- like immune response in various species (Gaulton et al, 1986), and induced T-cell immunity to reovirus type 3 in naive mice (Sharpe et al, 1984). In the poliovirus type II and rabies virus systems immunization with anti-id led to the production of virus-neutralizing antibodies (Reagan, 1985; Utdehaag and Osterhaus, 1985). A monoclonal anticlonotypic antibody to sendai virus recognizes the T-cell receptor of a virus- specific T-helper cell clone (Ertl and Finberg, 1984; Ertl et al, 1984). Immunization with the anticlonotypic antibody induces protective immunity against sendai virus infection by eliciting T-cell and B-cell immunity (Finberg and Ertl, (1986).

## ROLE OF MONOCLONAL ANTIBODIES IN THE STUDY OF BHV-1

The monumental achievement of hybridoma technology by Kohler and Milstein (1975) was the culmination of many discoveries by other workers (Goding, 1986). Of particular importance were the proof of the clonal selection theory (Burnet, 1957; Nossal and Lederberg, 1958), the development of cell fusion techniques (Okada, 1962; Littlefield, 1964), the artificial induction of plasmacytomas (Potter and Boyce, 1962), and finally the demonstration that it was possible to fuse two different plasma cell tumour lines with retention of both antibody products (Cotton and Milstein, 1973). Kohler and Milstein (1975) first produced monoclonal antibodies (Mabs) by fusing mouse plasmacytoma cells with normal splenic lymphocytes from a mouse that has been immunized with ovine erythrocytes. The fused plasmacytoma-lymphocyte cells (hybridomas) were grown in a medium which permitted only the growth of hybridoma cells. They found that several hybridomas secreted antibodies that specifically recognized an anitgenic determinant on ovine erythrocytes. As the technology has become widely used some technical advances have been made, but the basic procedure is essentially similar to the one described by Kohler and Milstein. The production of Mabs involves immunization of rats or mice with the desired antigen until they make a good antibody response. Spleens are removed and splenic lymphocytes are fused to a myeloma line with poly ethylene glycol (PEG). The fusion protocol results in a mixure of parental and hybrid cells. For selection of spleen-myeloma hybrids, the classic hyposanthine-aminopterin-thymidine (HAT) selection system of Littlefield (1964) is usually employed. The myeloma cells have been engineered with a genetic defect in the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT). HGPRT-negative myeloma cells can't use endogenous hypoxanthine to synthesize purines, and thus die in the presence of aminopterin, which blocks de novo synthesis of purines and pyrimidines. Parental spleen cells although not killed by aminopterin, will not grow in culture and eventually die. Only hybridomas, which inherit HGPRT activity and antibody production from spleen cells, and the ability to grow indefinitely from myeloma cells, will survive in HAT medium. Rapid and sensitive assays such as radioimmunoassay (RIA), and ELISA are used for screening hybridomas. Positive hybridomas once subcloned, can be grown in mass cultures or injected into syngeneic mice to generate high titer ascites.

Mabs are homogeneous populations of identical antibody molecules arising from a single B-cell clone. Since their initial description in 1975, Mabs have made a profound impact in all areas of biological research and biotechnology. High specificity and availability of murine Mabs have proven invaluable for expanding biological research in BHV-1. The use of Mabs have facilitated studies on defining biological activities associated with the viral proteins, identifying epitope maps on important structural proteins, and recognizing immunological intrrelationship between them.

Van Drunen Littel-van den Hurk et al (1984) produced and characterized a series of Mabs directed against BHV-1 glycoproteins (gps). He demonstrated that 180Kd gp is a dimer of 91Kd gp and also indicated that 74Kd gp and 55Kd gp, are components of a disulfied-linked complex, 130Kd gp. Initial studies on the biological activities of the gps using Mabs, showed that 130Kd\74Kd\55Kd, 180Kd\91Kd, and 71Kd gps participated to different extents in virus neutralization, and antibody and complement-mediated (ABC') cytolysis of virus-infected cells (Misra et al, 1982; Van Drunen Littel-van den Hurk et al, 1984). 130Kd\74Kd\55Kd, and 180Kd\91Kd gps were purified by immunoadsorbent chromatography using Mabs. The purified gps induced the production of antibodies, in rabbits, which neutralized viral infectivity, and in the presence of complemnt, lysed the virus-infected cells (Van Drunen Littel-van den Hurk and Babiuk, 1985). Using a panel of Mabs in a competition antibody binding assay, Van Drunen Littel-van den

Hurk et al, (1985) were able to identify 9 epitopes on the 180Kd\91Kd gp, of which 6 are involved in ABC' lysis. Seven epitopes on the 130Kd\74Kd\55Kd gp were identified, 6 of which are involved in neutralization, and 3 participate in ABC' lysis. Marshall et al (1986) identified BHV-1 gps which present on the virion's envelope and on the surface of virus-infected cells. In addition he described a third set of coprecipitating gps, 150Kd\77Kd, and showed it to be immunologically important. Other authers described Mabs with different specificities, and used them to study the biological activities of BHV-1 proteins (Collins et al, 1984; Chang et al, 1986, Okazaki et al, 1986; Trepanier et al, 1986).

Mabs to BHV-1 have been employed in radioimmunoprecipitation, and immunoblotting assays to analyse the antigenic relationship between BHV-1 isolates (Metzler et al, 1986; Friedli and Metzler, 1987).

In the field of diagnosis Collins et al (1985b, 1988) described the application of a rapid antigen-capture ELISA for the detection of BHV-1 using Mabs.

MANUSCRIPT

## ABSTRACT

A panel of murine monoclonal antibodies (Mabs) to bovine herpesvirus 1 (BHV-1) was prepared. These Mabs were directed against different glycoproteins (gps) on the virus, as determined by radioimmunoprecipitation. Three of them were neutralizing Mabs and reacted against 130/75/50Kd, 77Kd, and 97Kd gps respectively. A fourth non-neutralizing Mab recognized 97Kd gp. Each of the four Mabs reacted against a different epitope by compitition radioimmunoassay. The four Mabs were injected into rabbits for the production of anti-idiotypes antibodies (anti-id). Rabbit antisera containing maximum anti-idiotypic activity, as identified by ELISA, were purified by sequential immunoaffinity chromatography. The binding of BHV-1 Mabs to BHV-1 was inhibited by the respective anti-id using competitive ELISA. This inhibition suggested that, the anti-id were specific to the variable region of the antigen combining site of Mab. Absence of significant cross-reactivity among the anti-id for heterologous Mab indicated that the anti-id recognized unique determinants on the variable region of the homologous Mab.

# INTRODUCTION

Bovine herpesvirus type 1 (BHV-1) is an important pathogen of cattle producing many types of illnesses such as rhinotracheitis, conjunctivitis, pustular vulvovagintis, encephalomyelitis and fatal systemic infection (Kahrs, 1977). BHV-1 contributes considerably to the causes of bovine respiratory diseases (Jansen, 1980), and has been described as a significant component of the disease complex (Lupton and Reed, 1980). The disease presents a major economical problem in feedlot and dairy herds, and has been reported from most parts of the world. Attempts to control the disease by vaccination dates back to the work of Schwartz et al (1957), who developed the first live attenuated IBR vaccine by rapid passage in bovine cell cultures. Subsequently a number of live attenuated vaccines have been developed by adaptation to different culture systems (Schwartz et al, 1958; Zuscheck and Chow, 1961; Inaba, 1975; Bartha, 1974; Todd et al, 1971). Although the vaccines stimulated cell-mediated immunity (CMI) as well as antibody response to IBR (Gerber et al, 1978), many investigators have reported shedding of IBR virus from vaccinated animals which may serve as a source of infection to susceptible cattle (Kelling et al, 1973; Straub, 1976). Moreover, latency of vaccine strains in vaccinated animals doesn't prevent reinfection and latency of wild-type virus (Kahrs, 1976; Nettleton and Sharp, 1980; Pastoret et al, 1981). Temperature-sensitive (ts) vaccines have been prepared through selection of ts-mutant clones (Pastoret et al, 1980; Rossi and Kiesel, 1982), however latency may be established and virus shedding particularly in stressed animals may occur. The use of inactivated virus vaccines circumvented the problem of postvaccination reaction, latency, and reactivation of IBR virus (McKercher, 1971; Kelling et al, 1973; Wilson, 1974; Roberts et al, 1974), however these vaccines may not provide adequate protection against field exposure to BHV-1, and as a result mild disease may ensue with resulting viral latency (Frerichis et al, 1982).

In order to achieve the development of more efficient vaccines against herpesviruses, various modern biotechnological approaches are being actively explored. A novel approach is the production of idiotype vaccines. The network hypothesis of Neils Jerne (1974) provided an elegant concept for developing vaccines, in which anti-idiotype antibodies (anti-id) which represent internal images of the original antigen, may be used to prime or stimulate the immune system (Nisonoff and Lamoyi, 1981). The principle of idiotypic vaccination has been applied successfully for several viral systems (Gell and Moss, 1985; Kennedy et al, 1985; Reagan et al, 1983; Sharpe et al, 1984; Tanaka et al, 1986; Uytdehaag and Osterhaus, 1985; Gaulton et al, 1986).

This communication describes the production, purification, and characterization of anti-id to four different BHV-1 Mabs.

## MATERIALS AND METHODS

### Cells and viruses:

Madin-Darby bovine kidney (MDBK) cells were grown in minimum essential medium (MEM) (Gibco Laboratories, Grand Island, N.Y.) supplemented with 10% fetal bovine serum (FBS) (Hyclone Laboratories, Logan, Utah). The cooper-1 strain of BHV-1 obtained from American Type Culture Collection (Rockville, Md.) was used throughout the study.

### Virus purification:

Cells were infected with BHV-1 at a multiplicity of infection (MOI) of 0.10, maintained in MEM containing 5% FBS, and harvested when complete CPE was observed. Virus-infected cell were frozen at -70°C, thawed and clarified by centrifugation at 5000 Xg for 30 minutes. The supernatant was ultracentrifuged over a 3 ml cushion of 25% sucrose (w/v) in TEN buffer (0.01 M Tris hydrochloride, 0.001 M EDTA, 0.1 M NaCl, pH 7.2) at 25000 rpm in an SW27 rotor (Beckman Instrument, Inc. Fullerton, CA). The pellet was resuspended in TEN buffer to 1:60 of the original volume. The purified virus was sonicated (Sonic Dismembrator, model 300, Fisher) for 10 seconds and assayed by $TCID_{50}$ (Karber, 1931) and by plaque methods (Dulbecco, 1952).

### Monoclonal antibodies:

Balb\c mice were immunized with purified BHV-1 in PBS, inactivated by a 10-minute exposure to UV irradiation. Each mouse recieved three doses of $10^8$ $TCID_{50}$ of virus intraperitoneally at biweekly intervals. After one month, the mouse was boosted with the same preparation, and 4 days later, sacrificed. Fusion of splenocytes with mouse myeloma cells P3\X63\Ag8.653 was performed according to the methods described by Fazekas de St. Groth and Scheidegger (1980) and Molinaro and Nakamara (1982). Supernatants of growing hybridomas were screened for production of BHV-1-specific antibodies by ELISA. Subcloning of positive clones was performed in microtiter plates by using the limiting dilution method. Ascites fluids were obtained by intraperitoneal injection of $2X10^6$ to $5X10^6$ specific antibody-producing hybridoma cells into pristane (2,6,10,14-tetramethyl pentadecane, Aldrich Chemicals, Inc. Milwaukee)-primed Balb\c mice. Ascites fluid was collected and IgG was immediatetly purified on protein A-sepharose affinity column (Beckman Ins.). Subisotype specificities of Mabs were determined by using mouse Mab sub-isotyping kit (Hyclone Laboratories).

Neutralization assays:

Mab ascites fluids were diluted in MEM containing 5% FBS, and incubated with 50-100 pfu of virus for 1 hour at 37°C. Antibody-virus mixures were added to the confluent MDBK monolayers in petri dishes, adsorbed for 45 minutes, and followed by the addition of medium-agar mixure (1% purified agar in Dullecco MEM plus 10% FBS). After 3 days of incubation, monolayers were stained with neutral red for overnight before plaques were counted. Titers were expressed as reciprocals of the highest dilutions which reduced plaques by 50%. Neutralization assay using $TCID_{50}$ method was performed as described previously (Trepanier et al, 1986).

Radioimmunoprecipitation:

Confleunt monolayers of MDBK cells were inoculated with BHV-1 at MOI of 1 to 3. After adsorption of the virus for 1 hour, the monolayers were supplemented with methionine (met) - free MEM containing 2% FBS and incubated at 37°C. At 6 hours post infection (PI), 10 uCi of $S^{35}$-met (1094 CP/m mol, Du Pont Co, Wilmington, DE) per ml of met-free MEM was added to the culture. Cells were washed with PBS 24 hour PI, and solubilized in RIPA buffer (50 mM Tris, 500 mM NaCl, 5 mM EDTA, 50 um of PMSF, pH 7.4), 1% Triton X-100, and 0.1% SDS)). The cell lysates were left on ice for 15 minutes and clarified by centrifugation for 1 hour in a microfuge (Model 235B, Fisher Co.) at 4°C. Freshly prepared cell lysates (200 ul) were mixed with 10 ul of Mab ascites fluid and incubated overnight at 4°C. 50 ul of 10% protein A-sepharose CL-4B (Sigma chem. Co., St. Louis, MO) in RIPA buffer were added and incubated for an additional 2 hours. The sepharose beads were washed four-times with RIPA buffer (same as described previously except it contained 150 mM NaCl and no PMSF), resuspended in sample buffer (0.0625 M Tris-HCl [pH 6.8], 2.3 SDS, 10% glycerol, 5% ß-mercapto-ethanol, 0.00125% Bromophenol blue), boiled for 5 minutes, and analysed by SDS-PAGE and autoradiography.

Iodination of monoclonal antibodies:

The protein concentrations of purified Mabs were determined using Bio-Rad reagents (Bio-Rad Laboratories, Richmond, CA). Iodination of Mabs was done according to the methods of Markwell (1982). Briefly purified Mab in PBS at a concentration of 0.4 to 1.0 mg of protein per ml was incubated with 250 uCi of $NaI^{125}$ (ICN Radiochemicals, Irvine, CA) plus 2 iodo-beads (Pierce, Rockford, Illinois) for 15 minutes at room temperature. $I^{125}$-labeled Mab was separated by Excellulose GF-5 desalting column (Pierce), assayed by RIA and ELISA, and stored at -20°C until used.

Radioimmunoassay (RIA):

Polysterine microtiter plates (Immulon 2, Dynatech laboratories Inc, Alexandria, VA) were coated with $10^6$ $TCID_{50}$ of purified BHV-1 per ml of 0.05 M carbonate buffer, pH 9.6, for two hours at 37°C. Non-specific sites were blocked by incubating wells with 0.01 M PBS, pH 7.4, containing 0.05% Tween 20 and 1% horse serum (PBS-T-HS). Plates were washed with PBS-T and incubated for two hours at 37°C with 100 ul per well of several dilutions of each competitor Mab. Plates were washed and 100 ul of $I^{125}$-labeled Mab were added and incubated for 1 hour at 37°C. Plates were washed thoroughly, and radioactivity was measured by a gamma counter. The percentage competition was calculated as described by Kimura-Kuroda and

Yasui (1983), using the formula:

[100 (A-n)] / (A-B), where A is the count in the absence of competitor antibody, B is the count in the presence of homologous antibody, and n is the count in the presence of competitor antibody.

Production of anti-idiotypes:

Female New Zealand White rabbits were injected subcutaneously at multiple sites along the back, with 300 ug of protein A-sepharose-purified Mab emulsified in Freund's Complete Adjuvant. A second and a third intramuscular injections in Incomplete Freund's Adjuvant, and in PBS respectively, each containing 300 ug of protein, were given at biweekly intervals. Anti-Mab sera were collected periodically throughout the immunization schedule and analysed for anti-id activity by ELISA using purified Mab and purified normal mouse IgG coated plates. The coating concentrations were predetermined by checker-board titration. Sera were diluted in PBS-T, added to the plate and incubated for one hour at 37°C. Plates were washed and peroxidase-conjugated goat anti-rabbit IgG (Hyclone) was added to each well followed by substrate(2,2 azino-di [(3-ethylbenzthiazoline sulfonic acid)] (ABTS). Absorbance at 405 nm was measured using a Bio-Teck immunoasay reader.

Purification of anti-idiotypes:

The highest idiotype-specific activity was detected in serum samples collected two weeks after the initial injection, as determined by ELISA. Pooled rabbit serum of different samples at this period was purified and used throughout the study. To remove anti-mouse isotype and allotype antibodies, the rabbit anti-id serum was passed over an affinity column made by coupling normal Balb\c mouse IgG to Affi-gel 10 (Bio-Rad). The anti-id were passed through the column with 10 mM Tris-HCl, pH 7.4. Anti-mouse isotype and allotype antibodies were eluted with 0.1 M glycine buffer pH 2.5, and immediately neutralized with 1.0 M $NaCO_3$, pH 8.0. The effluent was further passed over protein A-sepharose column (Beckman) to isolate idiotype-specific IgGs.

Anti-idiotype inhibition assay:

Polyvinyle chloride microtiter plates were coated with 100 ul per well of $10^6$ $TCID_{50}$ of purified virus per ml. Plates were washed and blocked as described previously. Four-fold dilutions of anti-id or preimmunization sera in PBS-T-HS were incubated with equal volumes of diluted Mab and allowed to react for one hour at 37°C. The mixures were added to the wells and incubated for 30 minutes at 37°C. Bound Mab was detected with peroxidase-conjugated goat anti-mouse IgG. Controls consisted of PBS-T-HS and Mab preincubated with PBS-T-HS. Results were expressed as percentage inhibition of Mab binding, according to the following formula:

$$\frac{(A_{405} \text{ of Mab control}) - (A_{405} \text{ of sample mixure}) \times 100}{A_{405} \text{ of Mab control}}$$

# RESULTS

## Characteristics of monoclonal antibodies:

Initial screening by ELISA of the supernatants obtained from hybridomas, revealed 25 clones that reacted specifically with BHV-1. After further selection 12 stable cell lines producing Mabs were fully characterized. Antigen specificities of Mabs were determined by immunoprecipitation of $S^{35}$-met-labeled polypeptides from BHV-1-infected and mock-infected cells. Results of immunoprecipitation are illustrated for selected Mabs in Fig. 1 and summerized in table I. Three patterns were observed: one group of Mabs reacted against gps 130Kd, 75Kd, and 50Kd, a second group recognized gp 77Kd, and a third group precipitated gp 97Kd. None of the Mabs precipitated polypeptides from mock-infected cells.

Four Mabs were selected from this panel to produce anti-id in rabbits. The Mabs, 83, 111B, R54, and 38 were chosen because they reacted against immunologically important virus gps. Three of them (83, 111B, and R54) strongly neutralized viral infectivity, whereas 38 was non-neutralizing. Results of competition RIA between these Mabs showed that they were directed to different epitopes on the virus gps.

## Preparation of anti-idiotypes:

Each Mab was purified from ascites fluid and injected into a pair of rabbits to produce anti-id as described. The specificity of each hyperimmune serum to its immunizing Mab was monitored by ELISA throughout the immunization schedule. As represented by 111B Mab dose-response curve (Fig. 2), there is an initial increase in anti-id response after the first dose until it reaches a maximum about the second dose, after which it plateaus. The highest specific anti-id reaction occured 10 to 14 days after the first injection. A pool of this serum was purified as described previously. The success of the purification procedure was monitored by ELISA, and typical results were shown for anti-id 111B in Fig. 3. Before adsorption, the hyperimmune serum to 111B Mab reacted both with normal mouse IgG and 111B Mab, suggesting a reaction to common determinants (isotypes). Immunoglobulins which were eluted from the normal mouse affinity column reacted strongly with both antigens, indicating that it contained the anti-isotype and anti-allotype antibodies. In contrast the material which passed through the column reacted only with the Mab and therefore contained the anti-id.

In order to determine the presence of cross-reactive idiotopes among the Mabs used for the production of anti-id, each purified anti-id preparation was tested against homologous and heterologous Mab by ELISA. Table II shows that each anti-id was specific, with a high titer, to its corresponding immunizing Mab. To demonstrate that the specific response of each anti-id to its homologous Mab, is directed to the antigen-combining site of the Mab, an inhibition ELISA was conducted. As shown in Fig. 4, each anti-id inhibited the binding of its homologous Mab to BHV-1. The inhibition occured in a dose-dependent manner suggesting that each anti-id is directed against the antigen-combining site of the Mab.

## DISCUSSION

In the present study anti-idiotype antibodies were prepared against 4 different anti-BHV-1 Mabs with the intention of generating a population of internal image antibodies which would mimic an important antigenic determinant on the virus. Rabbit polyclonal anti-id antisera were produced following a xenogeneic immunization protocol, as it has been reported by several investigators that anti-id of xenogeneic origin are most immunogenic (Victor-Korbin, 1988). Data illustrated in Fig. 3 shows clearly the success of the procedure used for the purification of anti-id. Reactions of the purified anti-id against the normal mouse IgG were insignificant indicating the removal of contaminating anti-isotype and anti-allotype antibodies in the anti-id serum.

Table II reveals that the anti-id were highly specific for their homologous Mab which further indicated absence of cross-reactive idiotopes among the Mabs. This observation is supported by the fact that, the Mabs were found to bind different epitopes on the virus, as determined by competition RIA. Similar findings have been described by Reagan et al (1983). Each anti-id was found to inhibit the binding of its homologous Mab to the virus in a dose-dependent fashion (Fig. 4), suggesting that these anti-id contain populations of antibodies specific to the variable region of the Mab and most likely may mimic epitopes on the virus. The inhibition of Mab binding to the virus by anti-id have been described before, for the characterization of Herpes simplex virus anti-id (Lathey et al, 1986). Anti-id reagents which react specifically with the antigen-combining sites of antibodies, were successfully used to specifically prime or stimulate the immune system against a given antigen (Kennedy et al, 1986; Gaulton et al, 1986; Reagan, 1985), however immune suppression has also been reported (Cosenza and Kohler, 1972 Kearney et al, 1981).

BHV-1 Mabs used to produce anti-id in this study, recognized neutralizing epitopes on 130/75/50Kd, 97Kd, and 77Kd structural virus proteins. These glycoproteins were identified by several investigators to be involved in the immune recognition, attachment, and penetration of susceptible host cells (Van Drunen Littel-van den Hurk et al, 1984; and Babiuk, 1985; Chang et al, 1986). Marshall et al (1986) showed that 130/75/50Kd and 97Kd gps as important components of BHV-1 envelope and the surface of BHV-1-infected cells. Production of internal image anti-id to these gps would be of obvious benefit as vaccine candidates, as well as probes for cell receptors.

Figure 1: Polypeptide specificities of BHV-1 monoclonal antibodies. SDS-PAGE and autoradiography analysis of $S^{35}$-methionine-labeled polypeptides from mock-infected (lanes 3, 5, and 7), and BHV-1-infected (lanes 4, 6, and 8) cells. Cell lysates were precipitated with Mabs secreted by hybridomas 83 (lanes 3 and 4), 111B (lanes 5 and 6), and 38 (lanes 7 and 8). Lanes 1 and 2 show all $S^{35}$-methionine-labeled polypeptides from mock-infected (lane 1) and BHV-1-infected (lane 2) cell lysates.

# Immunoprecipitation of BHV-1 polypeptides by monoclonal antibodies

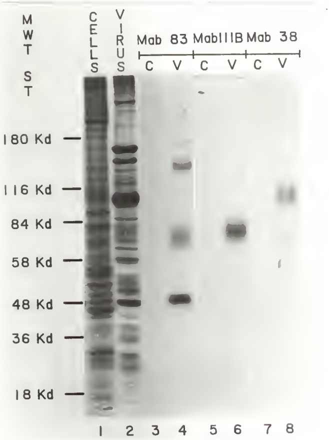

Figure 2: Anti-idiotype response in rabbits injected with Mab. Rabbits were immunized with 300 ug of purified Mab (111B) emulsified in Complete Freund's Adjuvant [ dose 1; subcut.], in Incomlete Freund's Adjuvant [dose 2; i/m], and in saline [dose 3; i/m]. Animals were bled at different time-intervals, and sera [1:10000] were tested against Mab (111B)-coated microtiter plates by ELISA.

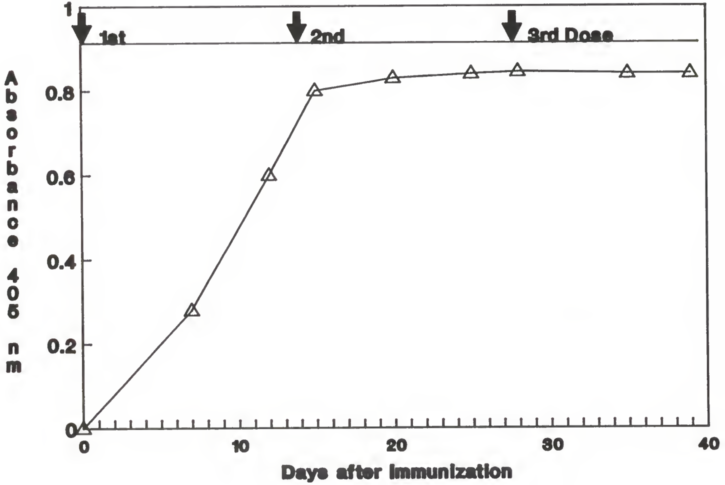
Anti-idiotype response in rabbits

Figure 3: Assay of the purification procedure of anti-idiotypes. Anti-id to 111B Mab was tested against 111B Mab (A), and normal mouse IgG (B), by ELISA. Plate coating concentrations were predetermined by checker-board titration. Serial dilutions of preimmunization serum, pool of primary immune response before adsorption with normal mouse affinity column, after adsorption, and material eluted from the affinity column were added to the plates in duplicate of wells. Bound antibodies were detected by addition of anti-rabbit peroxidase-conjugated IgG, followed by addition of substrate.

# Purification of anti-idiotypes

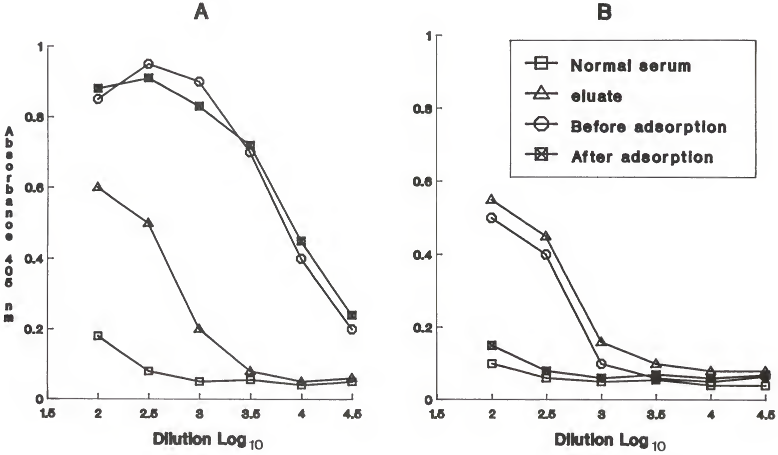

Figure 4: Analysis of anti-idiotypes by compitition ELISA. Microtiter polyvinyle plates were coated with a pretitrated amount of partially purified BHV-1 A limiting dilution of each Mab was incubated with four-fold serial dilutions of homologous purified anti-id or preimmunization IgG for 30 minutes at 37°C. The mixure was added to the plates in duplicates, and incubated for another 30 minutes at 37°C. Bound Mabs were detected by the addition of anti-mouse peroxidase-conjugated IgG, followed by addition of substrate. Percent inhibition was calculated according to the following formula:

$$\frac{[\ A_{405}\ \text{Mab control}] - [\ A_{405}\ \text{sample mixure}]}{A_{405}\ \text{of Mab control}} \text{ X100}$$

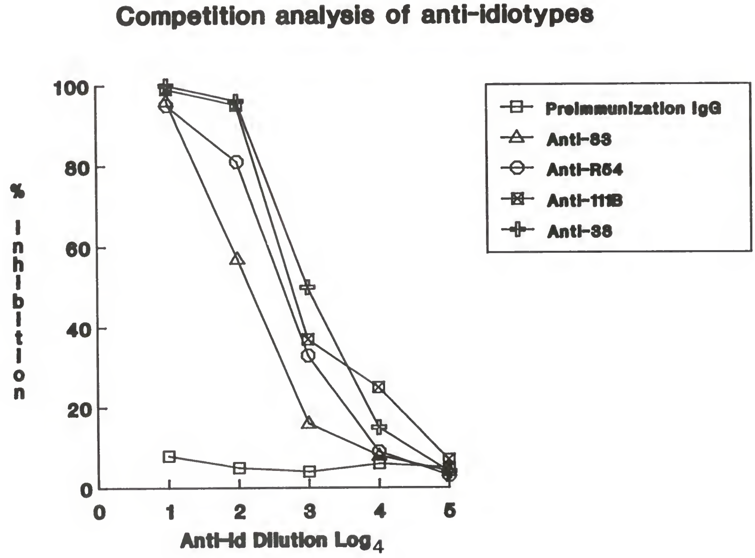
Competition analysis of anti-idiotypes
% Inhibition
100
80
60
40
20
0
0
1
2
3
4
5
Anti-Id Dilution Log4
Preimmunization IgG
Anti-83
Anti-R54
Anti-111B
Anti-38

Table I: Characteristics of BHV-1 monoclonal antibodies.

a. Mabs selected for the production of anti-id.

b. Subisotyping was determined by ELISA using a kit from Hyclone.

c. Virus-neutralization titers were determined by plaque reduction test using ascites fluid.

d. Titers were expressed as reciprocals of the highest dilutions which gave $A_{405}$ twice the blank.

## Characteristics of BHV-1 monoclonal antibodies

| Mab Designation | Protein Specificity | Isotype Specificity[b] | Virus Neutralisation Titer[c] | ELISA Titer[d] |
|---|---|---|---|---|
| 111B[a] | 77 Kd | IgG2a | 3000 | $10^6$ |
| 83[a] | 130/75/50 Kd | IgG3 | 10000 | $2X10^4$ |
| 38[a] | 97 Kd | IgG2a | 0 | $10^5$ |
| R54[a] | 97 Kd | IgG1 | 3000 | $10^5$ |
| 12 | 77 Kd | IgG2a | 100 | $10^5$ |
| 66 | 130/75/50 Kd | IgG2b | 0 | $10^5$ |
| 94 | 130/75/50 Kd | IgG1 | 0 | $10^4$ |
| 10B | 97 Kd | IgG2a | 0 | $10^5$ |
| 3 | 97 Kd | IgG2a | 0 | $10^5$ |
| 24 | 97 Kd | IgG2a | 100 | $10^5$ |
| 92 | 97 Kd | IgG1 | 100 | $10^4$ |
| 40 | 97 Kd | IgG2a | 0 | $10^4$ |

Table II: Reactivity of anti-idiotypes with homologous and heterologous Mab by ELISA. Predetermined concentrations of purified Mab and normal mouse IgG were used to coat plates. Purified anti-id were diluted to the same protein concentration. Serial dilutions of each anti-id were added to the plates. Titers of anti-id were expressed as reciprocals of the highest dilutions which gave absorbance greater than two-times the control.

## Reactivity of anti-idiotypes against homologous and heterologous monoclonal antibodies

| Mab | Purified anti-id IgG | | | |
|---|---|---|---|---|
| | Anti-111B | Anti-83 | Anti-38 | Anti-R54 |
| 111B | 60000 | <100 | 100 | <100 |
| 83 | <100 | 20000 | <100 | <100 |
| 38 | <100 | <100 | 20000 | <100 |
| R54 | <100 | <100 | <100 | 60000 |
| Normal moues IgG | <100 | <100 | <100 | <100 |

# REFERENCES

Bartha, A., 1974. Immunization of cattle with an attenuated IBR vaccine. Dev. Biol. Stand., 26:5-7.

Chang, L.W.S., Zee, Y.C., Pritchett, R.F., and Ardans, A.A., 1986. Neutralizing monoclonal antibodies directed to infectious bovine rhinotracheitis virus. Arch. Virol., 88:203-215.

Cosenza, H., and Kohler, H., 1972. Specific supression by antibodies to receptors. Proc. Natl. Acad. Sci., 69:2701-2704.

Dulbecco, R., 1952. Production of plaques in monolayer tissue cultures by single particles of an animal virus. Proc. Natl. Acad. Sci., 38:747-750.

Fazekas, de St. Groth, S., and Scheidegger, D., 1980. Production of monoclonal antibodies: Strategy and tactics. J. Immunol. Meth., 35:1-21.

Frerichis, G.N., Woods, S.B., Lucas, M.H., and Sands, J.J., 1982. Safety and efficacity of live and inactivated IBR vaccines. Vet. Rec., 111:116-122.

Gaulton, G.N., Sharpe, A.H., Chang,D.W., Fields, B.N., and Greene, M.I., 1986. Syngeneic monoclonal anti-idiotypes as prophylactic vaccines. J. Immunol., 137:2930-2936.

Gell, P.G.H., and Moss, P.A.H., 1985. Production of cell-mediated immune response to herpes simplex virus by immunization with anti-idiotypic heteroantisera. J. Gen. Virol., 66:1801-1804.

Gerber, J.D., Marron, A.E., and Kucera, C.J., 1978. Local and systemic cellular and antibody immune responses of cattle to IBR virus vaccine administered intranaslly or intramuscularly. Am. J. Vet. Res., 39:753-760.

Inaba, Y., 1975. Development of IBR live virus vaccine. J. Jpn. Vet. Med. Assoc., 28:410-414.

Jansen, S., Smart, J.N., and Nicholson, H.H, 1980. Observations on an outbreak of infectious bovine rhinotracheitis in a bull test station. Can. Vet. J., 21:24-27.

Jerne, N.K., 1974. Towards a network theory of the immune system. Ann. Immunol, (Paris), 125C:373-387.

Kahrs, R.F., 1976. Vaccination programs for bovine respiratory diseases. Mod. Vet. Prac., 57:29-32.

Kahrs, R.F., 1977. Infectious bovine rhinotracheitis : A review and update. JAVMA, 171:1055-1064.

Karber, G., 1931. Beitrag zur killektrien behandung pharmakologischer reihenversuche. Arch. Exp. Pathol. Pharmakol., 126:480-483.

Kearney, J.F., Barletta, R., Quan, Z.S., and Quintans, J., 1981. Monoclonal vs. heterogenous anti-H8 antibodies in the analysis of anti-phosphorylcholine response in Balb/c mice. Eur. J. Immunol., 11:877-881.

Kelling, C.L., Schipper, I.A., Strum, G.E., Carlson, R.B., and Tilton, J.E., 1973. Infectious bovine rhinotracheitis (IBR) abortion, observation on incidence in vaccinated and non-vaccinated and exposed cattle. Cornell Vet., 63:383-389.

Kennedy, R.C., 1985. Idiotype networks in hepatitis B virus infections. Curr. Top. Microbiol. Immunol., 119:1-13.

Kennedy, R.C., Melnick, J.L., and Dreesman, G.R., 1986. Anti-idiotypes and immunity. Sci. Amer., 255:48-56.

Kimura-Kuroda, J., and Yasui, 1983. Topographical analysis of antigenic determinants on envelope glycoprotein V3(E) of Japanese encephalitis virus, using monoclonal antibodies. J. Virol., 45:124-132.

Lathey, J.L., Rouse, B.T., Wiley, D.E., and Courtney, R.J., 1986. Production and characterization of anti-idiotypic antibody specific for a monoclonal antibody to glycoprotein D of herpes simplex virus. Immunol., 57:29-35.

Lupton, H.W., and Reed, D.E., 1980. Evaluation of experimental subunit vaccines for infectious bovine rhinotracheitis. Am. J. Vet. Res., 41:383-390.

Markwell, M.K., 1982. A new solid-state reagent to iodinate proteins I. Conditions for the efficient labeling of antiserum. Anal. Biochem., 125:427-432.

Marshall, R.L., Rodriguez, L.L., and Letchworth III., 1986. Characterization of envelope proteins of infectious bovine rhinotracheitis (Bovine Herpesvirus 1) by biochemical and immunological mehthods. J. Virol., 57:745-753.

McKercher, D.G., and Crenshaw, G.L., 1971. Comparative efficacity of intranasally and parentrally administered infectious bovine rhinotracheitis vaccines. JAVMA, 259:1362-1369.

Molinaro, C.A., and NaKamura, R.M., 1982. Production and clinical applications of monoclonal antibodies in the clinical laboratory. NaKamura, R.M., Dita, W.R., and Tucker, E.S., (eds), Immunol. Anal. Masson Pub., p. 129-143.

Nettleton, P.F., and Sharp, J.M., 1980. Infectious bovine rhinotracheitis virus excretion after vaccination. Vet. Rec., 107:379.

Nisonoff, A., and Lamoyi, E., 1981. Implications of the presence of an internal image of the antigen in anti-idiotypic antibodies: Possible application to vaccine production. Clin. Immunol. Immunopathol., 21:397-406.

Pastoret, P.-P., Babiuk, L.A., Misra, V., and Griebel, P., 1980. Reactivation of temperature-sensitive and non-temperature-sensitive infectious bovine rhinotracheitis vaccine virus with dexamethasone. Infec. Immunol., 29:483-488.

Pastoret, P.-P., Thiry, E., and Vindevoget, H., 1981. Problemes lies a la latence lors de la vaccination contre le virus de la rhinotracheite bovine infectieuse. Dev. Biol. Stand., 52:455-461.

Reagan, K.J., 1985. Modulation of immunity to rabies virus induced by anti-idiotypic antibodies. Curr. Top. Microbiol. Immunol., 119:15-30.

Reagan, K.J., Wunner, W.H., Wiktor, T., and Kopowski, H., 1983. Anti-idiotypic antibodies induce neutralizing antibodies to rabies virus glycoprotein. J. Virol., 48:660-666.

Roberts, A.W., Carter, G.R., and Carter, F.A., 1974. Infectious bovine rhinotracheitis virus recovered from a milk of a cow with mastitis. JAVMA, 164:413.

Rossi, C.R., and Kiesel, G.K., 1982. Effect of infectious bovine rhinotracheitis virus on viral shedding in challenge-exposed calves treated with dexamethasone. Am. J. Vet. Res., 43:1576-1579.

Schwartz, A.J.F., York, C.J., Zirbel, L.W., and Estella, L.A., 1957. Modification of infectious bovine rhinotracheitis (IBR) virus in tissue culture and development of a vaccine. Proc. Soc. Exp. Biol. Med., 96:453-458.

Schwartz, A.J.F., Zirbel, L.W., Estella, L.A., et al, 1958. Propagation and modification of infectious bovine rhinotracheitis (IBR) virus in porcine kidney tissue culture. Proc. Soc. Exp. Biol. Med., 97:680-683.

Sharpe, A.H., Gaulton, G.N., McDade, K.K., Fields, B.N., and Greene, M.I., 1984. Syngeneic monoclonal anti-idiotype can induce cellular immunity to reovirus. J. Exp. Med., 160:1195-1205.

Straub, O.C., 1976. Prophylactic measures against infectious bovine rhinotracheitis. Deutsch Tierarztliche Wochenschrift, 83 (6):270.

Tanaka, M., Sasaki, N., and Seto, A., 1986. Induction of antibodies against newcastle disease virus with syngeneic anti-idiotype antibodies in mice. Microbio. Immunol., 30:323-331.

Todd, J.D., Volence, F.J., and Paterson, I.M., 1971. Intranasal vaccination against IBR: Studies on early onset of protection and use of the vaccine in pregnant cows. JAVMA, 159:1370-1374.

Trepanier, P., Minocha, H.C., Bastein, Y., et al, 1986. Bovine herpesvirus 1: Strain comparison of polypeptides and identification of a neutralization epitope on the 90-kilodalton hemagglutinin. J. Virol., 60:302-306.

Uytdehaag, F.G.C., and Osterhaus, A.D.M.E., 1985. Induction of neutralizing antibody in mice against poliovirus type II with monoclonal anti-idiotypic antibody. J. Immunol., 134:1225-1229.

Van Drunen Littel-van den Hurk, S., Van Den Hurk, J.V., and Babiuk, L.A., 1985. Topographical analysis of bovine herpesvirus 1 glycoproteins: Use of monoclonal antibodies to identify and characterize fuctional epitopes. Virol., 144:216-227.

Van Drunen Littel-van den Hurk, S., Van Den Hurk, J.V., Gilchrist, J.E., Misra, V., and Babiuk, L.A., 1984. Interactions of monoclonal antibodies and bovine erpesvirus type 1 (BHV-1) glycoproteins: Characterization of their biochemical and immunological properties. Virol., 135:466-479.

Victor-Korbin, 1988. Regulatory function of anti-idiotype autoantibodies. In: Biological application of anti-idiotypes. vol. 1. Bona, C.A., (ed), CRC Press, Boca Raton, FA., p. 105-132.

Wilson, T.E., 1974. Observations and comments on two outbreaks of abortion associated with IBR viruses. Can. Vet. J., 15:227-229.

Zuscheck, R., and Chow, T.L., 1961. Immunogenicity of two infectious bovine rhinotracheitis vaccines. JAVMA, 139:236-237.

# APPENDIX

## DETAILED METHODOLOGY

### Virus purification

BHV-1-infected MDBK cultures were harvested, when maximum CPE was observed, frozen-thawed twice, and clarified by centrifugation in Sorval RC-5B at 5000 rpm for 30 minutes. All steps for virus purification were carried out at 4°C. Potassium tartarate solutions (w\v) were prepared in TEN buffer, pH 7.2. Clarified virus was concentrated 60X by using ultrafiltration cell (Amicon model 402 equiped with a 100000 M.W. cutoff membrane; Amicon, MA, USA). 0.5 ml of concentrated virus was layered onto a preformed 15% - 45% potassium tartarate gradint made as follows: 45% (4ml), 35% (2ml), 25% (2ml), and 15% (8ml), and ultracentrifuged, with low acceleration and low decceleration at 25000 rpm for 90 minutes in Beckman L5-65 (SW 27 rotor). 1 ml fractions were collected and tested for virus infectivity by $TCID_{50}$. Fractions showing high virus titers (6th - 8th fractions from bottom of the tube) were pooled, diluted in TEN buffer and ultracentrifuged at 25000 rpm for 5 hours to remove tartarate. The pellet was suspended in TEN buffer and stored at -70C° until plaque assayed.

### Determination of $TCID_{50}$

Tissue culture infectious dose 50 ($TCID_{50}$) was performed in 96 well microtiter plates. Monolayers of MDBK cell cultures were infected with 0.2 ml of serial log dilutions of stock virus (4 wells per dilution). The cultures were examined for CPE 48 - 72 hours post inoculation. $TCID_{50}$ was calculated according to the method of Karber (1931).

Protein determination

The protein concentration of purified virus, MAb, or rabbit IgG was determined by the Bio-Rad microassay method. Bovine plasma gamma globulin (BGG; 1.25 mg/ ml $DDH_2O$ stock solution) was used as standard protein, aliquated and stored at -20°C. The test was performed in 96 well microtiter plate (Dynatech Laboratories Inc, Alexandria, VA). 40 ul of concenterated dye reagent were placed in each well. The standard curve was determined by adding 10, 20, 30, upto 100ul of 0.1 mg/ml of BGG in duplicate of wells. Experimintal samples were diluted to match the standard in concentration, and added to wells. The volume content of each well was brought to 200 ul by sample buffer. After thorough mixing, absorbance at 600 nm was measured by EIA reader within 5 - 60 minutes. Wells containing only dye and buffer were used to blank the instrument.

Production of hybridomas

Spleen from previously immunized mouse was removed, placed in a petri dish, and washed in tow changes of 5 ml serum-free Dulbecco MEM (DMEM). The body of the spleen was massaged gently, pinched off at one end, and its contents were emptied into the medium. Spleen cells were mixed thoroughly, washed twice with DMEM by centrifugation at 1000 rpm for 10 minutes, and resuspended in 10 ml of serum-free DMEM. Myeloma cells (P3/X63/Ag8.653) were washed three times with DMEM by centrifugation at 1200 rpm for 10 minutes, counted, and resuspended in 10 ml of serum-free DMEM. Spleen cells ($2X10^8$) were mixed with $5X10^7$ myeloma cells (4:1 ratio). The mixure was centrifuged at 800 rpm for 10 minutes to form a loose pellet. Supernatant was discarded leaving enough liquid (0.2 ml) to loosen the pellet. 1 ml of warm 50% polyethylene glycol (PEG) was added to the pellet over a one-minute period with gentle tapping of the tube, and allowed to stand for 90 seconds. 10 ml of serum-free DMEM were then added over a five-minute period, and allowed to stand for 10 minutes. The content was gently mixed by pipetting five times to resuspend the cells. The cell suspension was centrifuged at 800 rpm for 10 minutes. The supernatant was discarded and the pellet was resuspended in DMEM plus 10% FBS, and centrifuged at 1000 rpm for 10 minutes. The pelleted cells were finally resuspended in 192 ml of HAT medium containing 20% FBS, and seeded in 24-well plates at a rate of 1 ml per well. The plates were previously seeded with $10^5$ macrophages per ml per well. Plates were covered with aliminum foil, and incubated at 37°C, 7% $CO_2$.

Immunoprecipitation

Confluent monolayers of MDBK cells, grown in 60X15 mm plates, were infected with BHV-1 at MOI of 1 to 2, and supplemented with 3 ml of medium made by mixing equal volumes of Methionine-free MEM, and MEM plus 5% FBS. At 6 hour post infection (PI), medium was aspirated, and cultures were washed once with PBS, pH 7.2. 4 ml of Met-free MEM plus 2% FBS, containing 5 - 10 uCi of ($S^{35}$)-Methionine per ml were added and incubated at 37C. 22 - 24 hour PI media were collected for virus concentration, cultures were washed with cold PBS, and lysed in 1 ml/dish of RIPA lysis buffer, pH 7.4. All steps of cell harvest were done on ice. Labeled cell lysates were frozen-thawed twice, left on ice for 15 minutes, and centrifuged for one hour in a microfuge. 200 ul of supernatant were incubated with 50 ul of polyclonal antisera, or 10 ul of MAb ascites, in polysterine microfuge tubes, overnight at 4°C with rotation. 50 ul of protein A sepharose CL-4B (10% w/v in RIPA washing buffer) were added to each tube and incubated for 2 hours with rotation at 4°C. The immune complexes were then washed in three changes of RIPA washing buffer (1 ml per wash) by centrifugation for 5 minutes at 4°C. After the final wash, supernatants were discarded and the pelleted complexes were resuspended in 100 ul SDS-sample buffer, pH 6.8, boiled for 5 minutes and centrifuged for 5 to 10 minutes. The supernatants were collected and used immediately, or stored at -20°C. The samples were boiled for 5 minutes prior to loading on the gel. After electrophoresis gels were dried, and visualized by autoradiography. An X-ray film (X-Omat AR, Kodak diagnostic film) was placed between the dried gel, and the screen in a film cassette. The cassette was folded, fastened, and clamped between two plywood boards.

Exposure was carried out at -70°C for 3 - 6 days.

### Iodination of proteins

The whole process of iodination was done in a certified exhaust hood. The protein concentration of purified MAb was determined. The content of 1000 uCi of $NaI^{125}$ was reconstituted in 0.5 ml of PBS, pH 7.2. 50 ug of purified MAb in 100 ul of PBS was placed in a microfuge tube. 500 uCi of $NaI^{125}$ plus 2 iodobeads (Pierce, Rockford, Ill) were added to the tube, and left to react for 15 minutes at room temperature. The reaction was ended by transferring the mixure (without beads) to an Excellulose GF-5 desalting column (Pierce), previously equilibrated with 10 ml of buffer. The reaction tube was washed with 150 ul of buffer and transferred to the column. 1% horse serum, as a carrier, was added to the mixure in the column. After removal of the 0.5 ml of buffer in the first wash, the $I^{125}$-labeled MAb was eluted in 1.5 ml of buffer in the second wash, and finally the column was washed with 2 ml of buffer to remove unreacted iodine . The three washes were assayed by RIA and ELISA. The $I^{125}$-MAb was stored at -20°C until used.

### Enzyme-linked immunosorbent assay (ELISA)

ELISA was used to analyse MAb and anti-id. Polyvinyle chloride microtiter plates (Dynatech lab.) were coated with 100 ul per well of antigen (partially purified virus, or MAb diluted in 0.05 M carbonate buffer, pH 9.6) for 1.5 hour at 37°C. The coating concentrations of virus, and MAb were predetermined by checkerboard titrations. Non-specific reactions were blocked by adding PBS containing 1% heat inactivated horse serum (250 ul) to each well, and incubating for 1 hour at 37°C. Plates were washed four times with PBS containing 0.05% Tween 20 (PBS-T) between each successive step. Test antibody diluted in PBS-T plus 1% horse serum (PBS-T-HS) was added to the wells. Bound antibody was detected by peroxidase-conjugated, affinity-purified, goat anti-mouse, or anti-rabbit IgG (Hyclone lab.) diluted in PBS-T plus 1% goat serum. Substrate, 2,2 azino-di- [(3-ethylbenz-thiazoline sulfonic acid)] (ABTS) plus 0.002 M $H_2O_2$ in 0.05 M citrate buffer at pH 4 was added to the wells, and incubated for 20 minutes at room temperature, then absorbance at 405 nm was measured using a Bio-Teck Immunoassay Reader. The titers were expressed as reciprocals of the highest dilutions which give absorbance two times greater than negative controls.

# PREPARATION OF REAGENTS

**ABTS** : Stock solution of (2,2'-Azino-Di (3-ethylbenzthiazoline sulfonic acid)

| | |
|---|---|
| Diamonium salt | 0.5487 g |
| Deionized distled water (DDi. $H_2O$) | 25 ml |

Cover container with aliminium foil, and store at 4°C. To prepare substrate solution add 125 ul of stock ABTS to 25 ml citrate buffer containing 100 ul hydrogen peroxide ($H_2O_2$).

**Agar** : 2% purified agar in DDi. $H_2O$ is autoclaved at 15 lbs. for 30 minutes. At 45°C water path, equal volumes of 2% agar, and 2X DMEM are mixed and used for plaquing.

**Antibiotics** : A stock solution (100 ml) contains:

| | |
|---|---|
| Penicillin G | $10^6$ i.u |
| Streptomycin | 1 g |
| Kanamycin | 50 mg |

Dissolve in sterile DDi. $H_2O$. One ml of stock solution is added to one liter of media (MEM, or RPMI). Geramycin (Gentamycin), at 0.25 ml per liter, is added to media prior filtration.

**Autoradiography**

**Developing solutions**:

Kodak GBX developer and replinisher: 103 ml in 370 ml DDi. $H_2O$

Stop solution: acetic acid 1:100 in DDi. $H_2O$

| | |
|---|---|
| Kodak fixer | 89.5 g in 500 ml DDi. $H_2O$ |

Store developer and fixer in dark containers at room temperature.

Developing procedure:

| | |
|---|---|
| GBX developer | 2 - 3 minutes |
| Stop solution | 1 minute |
| Fixer | 5 minutes |

Rinse with running tap water for 20 - 30 minutes and air dry.

### Buffers

Carbonate: 10X stock solution, pH 9.6

| | |
|---|---|
| $Na_2CO_3$ | 15.9 g |
| $NaHCO_3$ | 29.3 g |

Dissolve in one liter, and adjust pH with NaOH pellets. Working solution is prepared by diluting stock slution 1:10 in $DDiH_2O$, and used for coating antigens to ELISA plates.

Citrate buffer: for ABTS 45 mM, pH 4.0

| | |
|---|---|
| Citric acid | 9.5 g |
| DDi. $H_2O$ | 900 ml |

Dissolve and adjust pH to 4.0 with 1 N NaOH (about 60 ml). Q.S to one liter, autoclave at 15 lbs. for 15 minutes, and store at 4C.

### Phosphate buffered saline (PBS)

ELISA PBS: 10 mM, pH 7.4

| | |
|---|---|
| NaCl | 8.0 g |

| | |
|---|---|
| $KH_2PO_4$ | 0.2 g |
| KCl | 0.2 g |

plus one of the following three:

| | |
|---|---|
| $Na_2HPO_4$ (anhydrous) | 1.15 g |
| or $Na_2HPO_4 \cdot 7H_2O$ | 2.17 g |
| or $Na_2HPO_4 \cdot 12H_2O$ | 2.9 g |

Dissolve in one liter with DDi. $H_2O$, and use for ELISA or dialysis.

PBS: 250 mM, pH 7.2

Basic solutions:

Solution # 1:

$Na_2HPO_4 \cdot 7H_2O$ 134 g in a liter of DDi. $H_2O$.

Solution # 2:

$NaH_2PO_4 \cdot H_2O$ 69.1 g in a liter of DDi. $H_2O$.

Buffer stock: pH 7.15

| | |
|---|---|
| Solution # 1 | 386 ml |
| Solution # 2 | 114 ml |
| DDi. $H_2O$ | 500 ml |

PBS working solution, pH 7.2

| | |
|---|---|
| Buffer stock | 80 ml |
| NaCl | 17 g |
| DDi. $H_2O$ | 1920 ml |

autoclave at 15 lbs. for 15 minutes.

RIPA (Radioimmunoprecipitation assay) Buffer: pH 7.4

| | |
|---|---|
| 50 mM Tris-HCl | 0.605 g |
| 500 mM NaCl | 2.923 g |
| 1% Triton X-100 | 1.0 ml |
| 5 mM EDTA | 0.1901 ml |
| 0.1% SDS | 0.1 g |

Protease inhibitors are added at the rate of 50 ug for PMSF (Phenylmethyl sulfonylfluoride), and 10 ug/ml of Aprotinin. Dissolve in 100 ml of DDi. $H_2O$. This buffer is used for lysis of cells.

RIPA (washing buffer): pH 7.4 (100 ml DDi. $H_2O$)

| | |
|---|---|
| 10 mM Tris-HCl | 0.121 g |
| 150 mM NaCl | 0.877 g |
| 1% Triton X-100 | 1.0 ml |
| 1 mM EDTA | 0.03802 g |
| 1% Sodium deoxycholate | 1.0 g |
| 0.1% SDS | 0.1 g |

SDS- Sample Buffer, pH 6.8, 100 ml

| | |
|---|---|
| 62.5 mM Tris-HCl | 1.51 g |
| 10% glycerol | 10 ml |
| 5% B-mercapto-ethanol | 5 ml |
| 2.3% SDS | 2.3 g |
| 0.00125 % Bromophenol Blue | .00125 g |

Dissolve in 100 ml DDi. $H_2O$, and adjust pH 6.8 with HCl. Donot add base if too much acid is added. Store at 4°C.

TEN Buffer, pH 7.2; 10X stock solution .

| | |
|---|---|
| 10 mM Tris-HCl | 12.11 g |
| 1 mM EDTA | 3.72 g |
| 100 mM NaCl | 58.44 g |

Dissolve in 100 ml of DDi. $H_2O$, sterilize by filtration through 0.45 u filter. Store at 4°C.

Electrophoresis reagents:

Acrylamide stock (30%):

| | |
|---|---|
| 29.2% Acrylamide monomer | 29.2 g |
| 0.8% Methylene-bis-acrylamide | 0.8 g |
| DDi. $H_2O$ | 100 ml |

Dissolve by stirring for 1 hour at room temperature. Filter through Watman # 1 paper, cover with foil and store at 4°C.

Lower gel buffer, pH 8.8

| | |
|---|---|
| Tris-base | 36.34 g |
| SDS | 0.8 g |

Dissolve in 200 ml DDi. $H_2O$. Adjust pH with HCl, and donot add base if too much acid is added. Store at 4°C.

Upper gel buffer, pH6.8

| | |
|---|---|
| Tris-base | 6.06 g |
| SDS | 0.4 g |

Dissolve in 100 ml of DDi. $H_2O$, and adjust the pH to 6.8 in a similar way to lower gel buffer.

Running buffer, pH 8.3

| | |
|---|---|
| 25 mM Tris-base | 6.06 g |

| | |
|---|---|
| 192 mM glycine | 28.84 g |
| 0.1% SDS | 2.0 g |

Dissolve in 2 liters of DD. $H_2O$, pH doesnot require adjustment.

10% Slab Gel:

Lower Gel (Running Gel):

| | |
|---|---|
| Lower gel buffer | 6 ml |
| 30% acrylamide | 8 ml |
| DDi. $H_2O$ | 10 ml |
| 10% Ammonium persulfate | 40 ul |
| TEMED | 20 ul |

Degas for 10 minutes, add ammonium persulfate, and TEMED, and load.

Upper Gel (Stacking Gel):

| | |
|---|---|
| Upper gel bufer | 3 ml |
| 30% acrylamide | 1.8 ml |
| DDi. $H_2O$ | 7.2 ml |
| 10% ammonium persulfate | 40 ul |
| TEMED | 15 ul |

Treat in a similar way to upper gel.

Stain:

0.2% Coomassie brillant blue

50% Methanol

12% Glacial acetic acid

Make to one liter with DDi. $H_2O$. Cover gel completely with stain and rock for 4 hours. the stain is reusable.

Destain:

20% Ethanol

10% Glacial acetic acid

Make to one liter with DDi. $H_2O$.

HAT (Hypoxanthine Aminopterin Thymidine) Medium:

Stock Solutions:

HT (Hypoxanthine Thymidine): 100X

| | |
|---|---|
| Hypoxanthine | 0.1361 g |
| Thymidine | 0.0378 g |

Dissolve in 100 ml DDi. $H_2O$ by stirring at 45 - 50C for one hour, store at -20°C.

Aminopterin: 100X (4-amino-folic acid; 4-aminopteroyl glutamic acid)

| | |
|---|---|
| Aminopterin | 0.00176 g |

Add 90 ml of DDi. $H_2O$. To dissolve add 0.5 ml 1 N NaOH. After adjusting the volume to 100 ml, neutralize the solution by adding 0.5 ml 1 N HCl. Store at -20°C protected from light.

Glutamine: 100X

Dissolve 3 g in 100 ml of DDi. $H_2O$.

Sodium pyruvate: 100X

Dissolve 1.1 g in 100 ml of DDi. $H_2O$.

HAT (working medium):

To 500 ml of complete DMEM, add the following:

| | |
|---|---|
| HT stock solution | 5 ml |

| | |
|---|---|
| Aminopterin stock solution | 5 ml |
| Glutamine stock | 7.5 ml |
| Sodium pyruvate stock | 5 ml |
| Non-essential aminoacids MEM-100X | 5 ml |
| Vitamin solution MEM-100X | 5 ml |

Add the required serum, wrap bottle in foil, and store at 4°C.

Hydrogen peroxide($H_2O_2$): 3%, 500 mM

Add 1.7 ml to 100 ml DDi. $H_2O$. Cover with foil and store at 4°C.

Methionine-free MEM:

| | |
|---|---|
| 10X Eagles salt | 100 ml |
| 100X antibiotics | 10 ml |
| 100X $CaCl_2$ | 10 ml |
| 100X MEM vitamins | 10 ml |
| 100X glutamine | 10 ml |
| 50X aminoacids stock (without Met) | 20 ml |
| 10% glucose | 5 ml |
| Gentamycin | 0.25 ml |
| DDi. $H_2O$ | 835 ml |

Sterilize by filtration and store at 4°C.

Neutral red: 1% stock solution

Prepare in DDi. $H_2O$, dissolve by stirring at low heat for 30 to 60 minutes, filter through two pieces of Watman # 1 and # 2 papers, autoclave at 10 lbs. for 5 to 7 minutes, cover with foil and store at 4C. Use

1.2 ml per 100 ml agar-media mixure for plaquing.

Polyethylene glycol(PEG): 50% (w\v)

Melt 5 g of PEG (M.W 4000) and sterilize by autoclaving at 15 lbs. for 30 minutes, cool to 50C in a water bath, and combine with 5 ml of 0.2% glucose in PBS to form 50% PEG.

Trypsin: stock solution 0.25%

Dissolve 2.5 g trypsin powder in a liter of PBS, pH 7.2. Filter through 0.45 u filter, and store at -20°C.

Trypsin-EDTA:

| | |
|---|---|
| 0.25% trypsin stock | 20 ml |
| 10% EDTA | 0.5 ml |
| PBS, pH 7.2 | 30 ml |

Mix and store at -20°C.

# REAGENTS AND ADDRESSES

| | |
|---|---|
| ABTS (2,2'-Azino-Di (3-Ethyl Benz thiazoline Sulfonic Acid) Diammonium salt | Sigma, Chem. Co., St. Louis, MO. |
| Acrylamide | Polysciences, Inc. Warrington, PA. |
| Agar | Difco, Detroit, MI. |
| Aminopterin (4-amino-folic acid; 4-aminopteroyl glutamic acid) | Sigma, Chem. Co., St. Louis, MO. |
| Ammonium persulfate | Bio-Rad, Richmond, CA. |
| Citric acid | Fisher Scientific Co. Fair Lawn, NJ |
| Coomassie brillant blue R | Sigma, Chem. Co., St.Louis, MO. |
| EDTA (Ethylene Diamine Tetraacetic Acid) Sodium salt | Sigma, Chem. Co., St.Louis, MO. |
| Fetal bovine serum | Hazelton Res. Prod.Denver, PA. |
| Freund's adjuvants | Difco, Detroit, MI. |
| Glycerol | Amer. Scientific Prod. McGaw, IL. |
| Glycine | Polyscinces Inc, Warrington, PA. |
| Hepes | Sigma, Chem. Co., St. Louis, MO. |
| Hydrogen peroxide | Fisher Scientific Co., Fair Lawn,NJ |
| Hypoxanthine (6-hydroxypurine) | Sigma, Chem. Co., St. Louis, MO. |
| Iodine ($I^{125}$) ($NaI^{125}$) | ICN Radiochemicals Irvine, CA. |

| | |
|---|---|
| Kodak developer and replenisher<br>Fixer<br>X- Omat AR film (XAR-5) | East Kodak Co., Rochester, NY. |
| Mercaptoethanol | Sigma, Chem. Co., St. Louis, MO. |
| N,N'-Methylene-bisacrylamide | East Kodak Co., Rochester, NY. |
| MEM | KC- Biological Co. Lenexa, KS. |
| MOPS | Sigma, Chem. Co., St. Louis, MO. |
| Neutral red | Fisher Scientific Co., Fair Lawn, NJ |
| Non-Essential aminoacids MEM-100X | Irvine Scientific Santa Ana, CA |
| Polyethylene glycol (PEG) | Sigma, Chem. Co., St. Louis, MO. |
| Sodium bicarbonate ($NaHCO_3$) | Gibco Lab. Chagrin Falls, OH. |
| Sodium carbonate ($Na_2CO_3$) | Fisher Scientific Co., Fair Lawn, NJ |
| Sodium deoxycholate | Sigma, Chem. Co., St. Louis, MO. |
| Sucrose | Sigma, Chem. Co., St. Louis, MO. |
| Sulfur [35]-Methionine ($S^{35}$-Met) | Du Pont Co., Wilmington, DE. |
| TEMED | Bio-Rad Richmond, CA. |
| Thymidine | Nutr.Biochem.Cor. Cleavland, OH. |
| Tris (Trizma base) | Sigma, Chem. Co. St. Louis, MO. |
| Trypsin | Difco Detroit, MI. |

| | |
|---|---|
| Tween 20 | Fisher Scientific Co., Fair Lawn, NJ |
| Vitamins solution MEM-100X | Irvine Scientific Santa Ana, CA. |

# BIBLIOGRAPHY

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Ada, G.L., 1986. Progress towards better vaccines. Bell, R., and Torrigiani, G. (eds), Oxford University Press, p. 1.

Albinanti, F.K., and Plumer, G.J., 1961. The isolation of infectious bovine rhinotracheitis virus from cattle affected with conjunctivitis - Observation on experimental infection. Am. J. Vet. Res., 22:13-17.

Armstrong, J.A., Pereira, H.G., and Andrews, C.H., 1961. Observations on the virus of infectious bovine rhinotracheitis, and its affinity with the herpesvirus group. Virol., 14:276-285.

Arnon, R., and Sapira, M., 1984. Anti-infleunza synthetic vaccine. In: Modern approaches to vaccines. Lerner, R.A., and Chanock, R.M., (eds), Cold Spring Harbor, N.Y., p. 109-113.

Arsenakis, M., and Roizman, B., 1985. Genetic engineering of herpes simplex virus genomes. In: High technology route to virus vaccines. Dreesman, G.R., Bronson, J.G., and Kennedy, R.C., (eds), Proceedings of the 1st ann. southwest foundation for biomed. res., Washington, D.C., p. 75-81.

Assaf, R., Marsolais, G., Marois, P., and Payment, P., 1975. Correlation between the serum neutralization test and the indirect immunoflourescent test fot the detection of specific antibodies to IBR virus. Can. J. Comp. Fed., 39:224-226.

Babiuk, L.A., Bielefeldt Ohmann, H., Gifford, G., Czarniek, C.W., Scialli, V.T., and Hamilton, E.B., 1985. Effect of bovine alpha 1 interferon on bovine herpesvirus type 1-induced respiratory disease. J. Gen. Virol., 66:2383-2394.

Babiuk, L.A., Wardley, R.C., and Rouse, B.T., 1975. Defense mechanisms against bovine herpesvirus: Relationship of virus-host cell events to susceptibility to antibody-complement cell lysis. Infect. Immunol., 12:958.

Bagust, T.J., 1972. Comparison of biological, biophysical, and antigenic properties of four strains of infectious bovine rhinotracheitis herpesvirus. J. Comp. Pathol., 82:365-374.

Baker, J.A., McEntee, K., and Gillespie, J.H., 1960. Effects of infectious bovine rhinotracheitis-infectious pustular vulvovaginitis (IBR-IPV) virus on newborn calves. Cornell Vet., 50:156-170.

Bartha, A., 1974. Immunization of cattle with an attenuated IBR vaccine. Dev. Biol. Stand., 26:5-7.

Berrios, P.E., and McKercher, D.G., 1975. Characterization of caprine herpesvirus. Am. J. Vet. Res., 36:1755-1762.

Binz, H., and Wigzell, H., 1978. Induction of specific immune unresponsiveness with purified mixed leukocyte culture-activated T-lymphocytes as autoimmunogens III. Proof for the existance of autoanti-idiotypic killer T cells, and transfer of suppression to normal syngeneic recepients by T or B lymphocytes. J. Exp. Med., 147:63-76.

Bishop, G.A., Marlin, S.D., Schwartz, S.A., and Glorioso, J.C., 1984. Human natural killer cell recognition of herpes simplex virus type-1 glycoproteins specificity analysis with use of monoclonal antibodies and antigenic variants. J. Immunol., 133:2206-2214.

Bittle, J.L., Houghten, R.A., Alexander, H., Shinnick, T.M., Sutcliffe, J.G, Lerner, R.A., Rowlands, D.J., and Brown, F., 1982. Protection against foot-and-mouth disease by immunization with a chemically synthesized peptide predicted from the viral nucleotide sequence. Nature, 298:30-33.

Bittle, J.L., Worrell, P., Houghten, R.A., Lerner, R.A., Rowlands, D.J., and Brown, F., 1984. Immunization against foot-and-mouth disease with a chemically synthesized peptide. In: Modern approaches to vaccines. Lerner, R.A., and Channock, R.M., (eds), Cold Spring Harbor, N.Y., p. 103-107.

Bocciarelli, D.S., Osfei, Z., Mondino, G., and Persechino, A., 1966. The core of a bovine herpesvirus. Virol. 30:58-61.

Bolton, D.C., Zee, Y.C., and Ardans, A.A., 1983. Identification of envelope and nucleocapsid proteins of infectious bovine rhinotracheitis virus by SDS-poly acrylamide gel electrophoresis. Vet. Microbiol., 8:57-68.

Bommeli, W.R, and Kihm, U., 1982. ELISA the nucleus of the IBR/IPV control program in Switzerland. Curr. Top. Vet. Med. Anim. Sci., 22:242-251.

Bommeli, W.R., Khim, U., Lazarowicz, M., and Steck, F., 1980. Rapid detection of antibodies to infectious bovine rhinotracheitis (IBR) virus by micro-enzyme-linked immunosorbent assay (Micro ELISA). Proc. 2 Int. Symp. Vet. Lab. Diagnost., 235-239.

Bona, C.A, 1985. Epibodies: a particular set of anti-idiotypes specific for autoantibodies. Clin. Immunol., (Newsl.) 6:87-89.

Bona, C.A., Lieberman, R.,Chien, C.C, Mond, J., House, S., Green, I., and Paul, W.E., 1978. Immune response to levan. I. Kinetics and ontogeny of anti-levan and anti-insulin antibody response and expression of cross-reactive idiotypes. J. Immunol., 120:1436-1442.

Bona, C.A., and Paul, W.E., 1979. Cellular basis of expression of idiotypes. I. Supressor cells specific for MOPC 460 idiotype regulate the expression of cells secreting anti-trinitrophenyl antibodies bearing 460 idiotype. J. Exp. Med., 149:592-600.

Bottomly, K., and Mosier, D.E., 1979. Mice whose B cells cannot produce the T15 idiotype also lack an antigen-specific helper T cells required for T15 expression. J. Exp. Med., 150:1399-1408.

Bruck, C., Co M-S, Slaoui, M., Gaulton, G.N., Smith, T., Fields, B.N., Mullins, J.I., and Greene, M.I., 1986. Nucleic acid sequence of an internal image bearing monoclonal anti-idiotype and its comparison to that of the external antigens. Proc. Natl. Acad. Sci., 83:6578-6582.

Burnet, F.M., 1957. A modification of Jerne's theory of antibody production using the concept of clonal selection. Austral. J. Sci., 20:67-69.

Bush, C.E., and Pritchett, R.F., 1985. A comparison of the genome of bovine herpesvirus type 1 and pseudorabies virus. J. Gen. Virol., 66:1811-1817.

Campos, M., and Rossi, C.R., 1985. Cell-mediated cytotoxicity of bovine mononuclear cells to IBRV-infected cells: Dependence on Sephadex G-10 adherent cells. Vet. Immunol. Immunopathol., 8:363-375.

Carmichael, L.E., and Barnes, F.D., 1961. The relationship of infectious bovine rhinotracheitis virus to equine rhinopneumonitis virus. Proc. U.S. Livestock Sanit. Assoc., 65:384-388.

Carrillo, B.T., Ambrogi, A., Schudel, A.A, Vasquez, M., Dahme, E., and Pospischil, A., 1983. Meningoencephalitis caused by IBR virus in calves in Argentina. Zbl Vet. Med. B., 30:327-332.

Carter, V.C, Schaffer, P.A., and Tevethia, S.S., 1981. Theinvolvement of herpes simplex type 1 glycoproteins in cell-mediated immunity. J. Immunol., 126:1655-1660.

Chang, L.W.S., Zee, Y.C., Pritchett, R.F., and Ardans, A.A., 1986. Neutralizing monoclonal antibodies directed to infectious bovine rhinotracheitis virus. Arch. Virol., 88:203-215.

Chow, T.L., Mollelo, J.A., and Owen, N.V., 1964. Abortion experimentally induced in cattle by infectious bovine rhinotracheitis virus. JAVMA, 144:1005-1007.

Collins, J.K., Ayers, V.K., and Carman, J., 1988. Evaluation of an antigen-capture ELISA for the detection of bovine herpesvirus type 1 shedding from feedlot cattle. Vet. Microbiol., 16:101-107.

Collins, J.K., Bulla, G.A., Riegel, C.A., and Butcher, A.C., 1985c. A single dilution enzyme-linked immunosorbent assayfor the quantitative detection of antibodies to bovinherpevirus type 1. Vet. Microbiol., 10:133-147.

Collins, J.K., Butcher, A.C., and Fountain, A.F., 1986. A one-step competitive serologic ELISA for detecting neutralizing antibodies to bovine herpesvirus type 1. 28th Ann. Proc. Am. Assoc. Vet. Lab. Diagnost., Tucson, A.Z., p. 361-368.

Collins, J.K., Butcher, A.C., and Riegel, C.A., 1985a. Immune response to bovine herpesvirus type 1 infections: Virus-specific antibodies in sera from infected animals. J. Clin. Microbiol., 21:546-552.

Collins, J.K., Butcher, A.C., Riegel, C.A., McGrane, V., Blair, C.D., Teramoto, Y.A., and Winston, S., 1984. Neutralization determinants defined by monoclonal antibodies on polypeptides specified by bovine herpesvirus 1. J. Virol., 52:403-409.

Collins, J.K., Butcher, A.C., Teramoto, Y.A., and Winston, S., 1985b. Rapid detection of bovine herpesvirus type 1 antigens in nasal swab specimens with an antigen-capture ELISA. J. Clin. Microbiol., 21:375-380.

Cosenza, H., 1976. Detection of anti-idiotype reactive cells in the response to phosphocholine. Eur. J. Immunol., 6:114.

Cosenza, H., and Kohler, H., 1972. Specific supression by antibodies to receptors. Proc. Natl. Acad. Sci., 69:2701-2704.

Cotton, R.G.H., and Milstein, C., 1973. Fusion of twimmunoglobulin-producing myeloma cells. Nature, 244:42-43.

Cremer, K.J., Mackett, M., Wohlenberg, C., Notkins, A.L., and Moss, B., 1985. Vaccinia virus recombinant expressing herpes simplex virus type 1 glycoprotein D prevents latent herpes in mice. Science, 228:737-740.

Cummins, J.M., and Rosenquist, B.D., 1982. Temporary protection of calves against adenovirus infection by nasal secretion of interferon induced by IBR virus. Am. J. Vet. Res., 43:955-951.

Darcel, C.L., and Kozeib, S.C., 1984. The effect of kaolin adsorption of serum on the virus neutralization and enzyme-linked immunosorbent assays of antibody to bovine herpesvirus 1. J. Biol. Sand., 12:87-92.

Derbyshire, J.B., and Caplan, B.A., 1976. The isolation and characterization of a strain of infectious bovine rhinotracheitis virus from stillbirth in swine. Can. J. Comp. Med., 40:252-256.

Ditcher, M.A., Weiner, H.L., Fields, B.N., Mitchell, G., Noseworthy, J., Gaulton, G., and Greene, M.I., 1986. Anti-idiotypic antibody to reovirus binds to neurons and protects from viral infection. Ann. Neurol., 19:555-558.

Dietzschold, B., Cohen, G., Eisenberg, R., and Varrichio, A., 1984. Synthesis of an antigenic determinant of the herpes simplex virus (HSV) glycoprotein D which stimulate the production of neutralizing antibodies and which confers protection against a lethal challenge of HSV. In: Modern approaches to vaccine. Lerner, R.A., and Chanock, R.M., (eds), Cold Spring Harbor, N.Y., p. 146-149.

Dulbecco, R., 1952. Production of plaques in monolayer tissue cultures by single particles of an animal virus. Proc. Natl. Acad. Sci., 38:747-750.

Edman, J.C., Hallwell, R.A., Valenzuela, P., Goodman, H.M., and Rutter, W.J., 1981. Synthesis of hepatitis B surface and core antigens in E.coli. Nature, 291:503-506.

Emtage, J.S., Tacon, W.C.A., Catlin, G.H., Jenkins, B., Porter, A.G., and Garey, N.H., 1980. Influenza antigenic determinants are expressed from hemagglutinin genes cloned in Escherichia coli. Nature, 283:171-174.

Ertl, H.C., and Finberg, R.W., 1984. Sendai virus-specific T-cell clones: Induction of cytolytic T-cells by an anti-idiotypic antibody directed against a helper T-cell clone. Proc. Natl. Acad. Sci., 81:2850-2854.

Ertl, H.C., Homans, E., Tournas, S., and Finberg, R.W., 1984. Sendai virus-specific T-cell clones. V. Induction of a DTH response using an anti-T-cell anti-idiotypic antibody. J. Exp. Med., 150:1720-1727.

Evans, D.L., Barnett, J.W., Bower, J.M., and Dmochowski, L., 1972. Antigenic relationship between the herpesviruses of IBR, Marek's disease, and Burkitt's lymphoma. J. Virol., 10:277-287.

Fazekas, de St. Groth, S., and Scheidegger, D., 1980. Production of monoclonal antibodies: Strategy and tactics. J. Immunol. Meth., 35:1-21.

Finberg, R.W., and Ertl, H.C., 1986. Use of T-cell-specific anti-idiotypes to immunize against viral infections. Immunol. Rev., 90:122-155.

Finberg, R.W., and Ertl, H.C., 1987. The use of anti-idiotypic antibodies as vaccines against infectious agents. Crit. Rev. Immunol., 7:269-284.

Florent, G., and de Marneffe, C., 1986. Enzyme-linked immunosorbent assay used to monitor serum antibodies to bovine respiratory disease viruses. Vet. Microbiol., 11:309-317.

Forman, A.J., Babiuk, L.A., Misra, V., and Baldwin, F., 1982. Susceptibility of bovine macrophages to infectious bovine rhinotracheitis virus infection. Infec. Immunol., 35:1084-1057.

Francotte, M., and Urbain, J., 1984. Induction of anti-tobacco mosaic virus antibodies in mice by rabbit anti-idiotypic antibodies. J. Exp. Med., 160:1485-1494.

French, E.L., 1962. Relationship between infectious bovine rhinotracheitis (IBR) virus, and a virus isolated from calves with encephalitis. Aust. Vet. Sci., 38:555-556.

Frerichis, G.N., Woods, S.B., Lucas, M.H., and Sands, J.J., 1982. Safety and efficacity of live and inactivated IBR vaccines. Vet. Rec., 111:116-122.

Friedli, and Metzler, A.E., 1987. Reactivity of monoclonal antibodies to proteins of a neurotropic bovine herpesvirus 1 (BHV-1) strain and to proteins of representative BHV-1 strains. Arch. Virol., 94:100-122.

Gaulton, G.N., Sharpe, A.H., Chang,D.W., Fields, B.N., and Greene, M.I., 1986. Syngeneic monoclonal anti-idiotypes as prophylactic vaccines. J. Immunol., 137:2930-2936.

Gell, P.G.H., and Moss, P.A.H., 1985. Production of cell-mediated immune response to herpes simplex virus by immunization with anti-idiotypic heteroantisera. J. Gen. Virol., 66:1801-1804.

Gerber, J.D., Marron, A.E., and Kucera, C.J., 1978. Local and systemic cellular and antibody immune responses of cattle to IBR virus vaccine administered intranaslly or intramuscularly. Am. J. Vet. Res., 39:753-760.

Gerin, J.L., Purcell, R.H., and Lerner, R.A., 1984. Recombinant DNA and synthetic peptide approaches to HBV vaccine development: Immunogenicity and protective efficacy in chimpanzees. In: Modern approaches to vaccines. Lerner, R.A., and Chanock, R.M., (eds), Cold Spring Harbor, N.Y., p. 121-125.

Gibbs, E.P.J., and Rweyemamu, M.M., 1977. Bovine herpesvirus 1. Vet. Bull., 47:317-343.

Gillespie, J.H., Robson, D.S., Scott, F.W., and Schiff, E.I., 1985. In vitro protective effect of bacteria-derived bovine alpha interferon II against selected bovine viruses. J. Clin. Microbiol., 22:912-914.

Gillespie, J.H., Scott, F.W., Geissinger, C.M., Czarniecki, C.W., and Scialli, V.T., 1986. Levels of interferon in blood serum and toxicity studies of baceria-derived bovine alpha-1 interferon in dairy calves. J. Clin. Microbiol., 24:240-244.

Glorioso, J., Schroder, C.H., Kumel, G., Szczesiul, M., and Levine, M., 1984. Immunogenicity of herpes simplex virus glycoproteins gC and gB and their role in protective immunity. J. Virol., 50:805-812.

Goding, W.J., 1986. Monoclonal antibodies: Principles and practice. Production and application of monoclonal antibodies in cell biology, biochemistry, and immunology. Goding, J.W., (ed), Acad. Press, Orlando, FA., p. 2.

Green, N., Alexander, H., Olson, A., Alexander, S., Shinnic, T.M., Sutcliffe, J.G., and Lerner, R.A., 1982. Immunogenic structure of the infleunza virus hemoagglutinin. Cell, 28:477-487.

Grewal, A.A., Rouse, B.T., and Babiuk, L.A., 1980. Mechanisms of recovery from viral infections: Destruction of infected cells by neutrophils and complement. J. Immunol., 124:312-319.

Grzyck, J.M., Capron, M., Lambert, P.H., Dissons, C., Torres, S., and Capron, A., 1985. An anti-idiotypic vaccine against experimental schistosomiasis. Nature, 316:74-76.

Hall, W.T.K., Simmons, G.C., French, E.L., Snowdon, W.A., and Asdell, M., 1966. The pathogenesis of encephalitis caused by the infectious bovine rhinotracheitis virus. Austral. Vet. J., 42:229-237.

Herbert, C.N., Edwards, S., Bushnell, S., Jones, P.C., and Perry, C.T., 1985. Establishment of a statistical base for use of ELISA in diagnostic serology for infectious bovine rhinotracheitis. J. Biol. Stand., 13:243-253.

Hiernaux, J.R., 1988. Idiotypic vaccines and infectious diseases. Infection and immunity, 56:1407-1413.

Homan, E.J., and Easterday, B.C., 1981. Further studies of naturally occuring latent bovine herpesvirus infections. Am. J. Vet. Res., 42:1811-1183.

Honess, R.W., and Watson, D.H., 1977. Unity and diversity of herpesviruses. J. Gen. Virol., 37:15-37.

House, J.A., and Baker, J.A., 1971. Bovine herpesvirus IBR-IPV. The antibody virus neutralization reaction. Cornell Vet., 61:320-335.

Inaba, Y., 1975. Development of IBR live virus vaccine. J. Jpn. Vet. Med. Assoc., 28:410-414.

Jansen, S., Smart, J.N., and Nicholson, H.H, 1980. Observations on an outbreak of infectious bovine rhinotracheitis in a bull test station. Can. Vet. J., 21:24-27.

Jayarman, S., and Bellone, C.J., 1982. Hapten-specific responses to the phenyltrimethyl aminohapten III. Mice whose DTH responses cannot be abrogated by the presence of anti-idiotypic suppressor T cells lack a critical modulatory T cell function. J. Exp. Med., 155:1810-1822.

Jerne, N.K., 1974. Towards a network theory of the immune system. Ann. Immunol, (Paris), 125C:373-387.

Jerne, N.K., Rolland, J., and Cazenave, P.A., 1982. Recurrent idiotypes and internal images. Eur. Molec. Biol. Organ., 1:243-247.

Kahrs, R.F., 1976. Vaccination programs for bovine respiratory diseases. Mod. Vet. Prac., 57:29-32.

Kahrs, R.F., 1977. Infectious bovine rhinotracheitis : A review and update. JAVMA, 171:1055-1064.

Kaminjolo, J.S., Jr., and Gicho, J.N., 1972. Das Auftreten Von AntiKorpern gegen das virus der rhinotracheitis (IBR-virus) bei Rindern in Kenya. Tierartz Umsch., 27:111-112.

Kang, C-Y., and Kohler, H., 1986. Immunoglobulins with complementary paratope and idiotope. J. Exp. Med., 163:787-796.

Karber, G., 1931. Beitrag zur killektrien behandung pharmakologischer reihenversuche. Arch. Exp. Pathol. Pharmakol., 126:480-483.

Kearney, J.F., Barletta, R., Quan, Z.S., and Quintans, J., 1981. Monoclonal vs. heterogenous anti-H8 antibodies in the analysis of anti-phosphorylcholine response in Balb/c mice. Eur. J. Immunol., 11:877-881.

Kelling, C.L., Schipper, I.A., Strum, G.E., Carlson, R.B., and Tilton, J.E., 1973. Infectious bovine rhinotracheitis (IBR) abortion, observation on incidence in vaccinated and non-vaccinated and exposed cattle. Cornell Vet., 63:383-389.

Kendrick, J.W., Gillespie, J.H., and McEntee, K., 1958. Infectious pustular vulvovaginitis of cattle. Cornell Vet., 48:458-495.

Kennedy, R.C., 1985. Idiotype networks in hepatitis B virus infections. Curr. Top. Microbiol. Immunol., 119:1-13.

Kennedy, R.C., and Dreesman, C.R., 1984. Enhancement of the immune response to hepatitis B surface antigen: In vivo administration of anti-idiotype induces anti-HBs that express a similar idiotype. J. Exp. Med. 159:655-665.

Kennedy, R.C, Eichberg, J.E., Landford, R.E., and Dreesman, G.R., 1986a. Anti-idiotypic vaccine for type B viral hepatitis in chimpanzees. Sceince, 232:220-223.

Kennedy, R.C., Melnick, J.L., and Dreesman, G.R., 1986b. Anti-idiotypes and immunity. Sci. Amer., 255:48-56.

Kimura, H., and Wilson, D.B., 1984. Anti-idiotypic cytotoxic T cells in rats with graft vs. host disease. Nature, 308:463-464.

Kimura-Kuroda, J., and Yasui, 1983. Topographical analysis of antigenic determinants on envelope glycoprotein V3(E) of Japanese encephalitis virus, using monoclonal antibodies. J. Virol., 45:124-132.

Kirby, F.D., Martin, H.T., and Oster, D.C., 1974. An indirect HA test for the detection and assay of antibody to infectious bovine rhinotracheitis virus. Vet. Rec., 94:361-362.

Kleid, D.G., Yansura, D., Small, B., et al., 1981. Cloned viral protein vaccine for foot-and-mouth disease: Response in cattle and swine. Sceince, 214:1125-1129.

Kohler, G., and Milstein, C., 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature, 256:495-497.

Lambert, P.H., 1986. Anti-idiotype vaccines. In: Progress towards better vaccines. Bell, R., and Torrigiani, G., (eds), Oxford University Press., p. 89-96.

Lancaster, F., Chui, Y.L., and Batchelor, J.R., 1985. Anti-idiotypic T cells supress rejection of renal allografts in rats.Nature, 315:336-337.

Langbeheim, H., Arnon, R., and Sela, M., 1976. Effect on MS-2 coliphage obtained with a synthetic antigen. Proc. Natl. Acad. Sci., 73:4636-4640.

Lathey, J.L., Rouse, B.T., Wiley, D.E., and Courtney, R.J., 1986. Production and characterization of anti-idiotypic antibody specific for a monoclonal antibody to glycoprotein D of herpes simplex virus. Immunol., 57:29-35.

Little, S.P., Jofre, J.T., Courtney, R.J., and Schaffer, P.A., 1981. A virion-associated glycoprotein essential for infectivity of herpes simplex type 1. Virol., 115:149-160.

Littlefield, J.W., 1964. Selection of hybrids from matings of fibroblasts in vitro and their presumed recombinants. Science, 145:709-710.

Lupton, H.W., and Reed, D.E., 1980. Clearing and shedding of infectious bovine rhinotracheitis virus from the nasal mucosa of immune and non-immune calves. Am. J. Vet. Res., 41:117-119.

Lupton, H.W., and Reed, D.E., 1980. Evaluation of experimental subunit vaccines for infectious bovine rhinotracheitis. Am. J. Vet. Res., 41:383-390.

Mackett, M., Smith, G.L., and Moss, B., 1982. Vaccinia virus: a selectable eukaryotic cloning and expresssion vector. Proc. Natl. Acad. Sci. 79:7415-7419.

Mackett, M., Smith, G.L., and Moss, B., 1984. A general method for the production and selection of infectious vaccinia virus recombinants expressing foreign genes. J. Virol., 49:856-864.

Mackett, M., Yilma, T., Rose, J.K., and Moss, B., 1985. Vaccinia virus recombinants express vesicular stomatitis genes and protectively immunize mice and cattle. Science, 227:433-435.

Mac Laughlin, N.J., and Rosenquist, B.D., 1982. Duration of protection of calves against rhinovirus challenge exposure by infectious bovine rhinotracheitis virus-induced interferon in nasal secretions. Am. J. Vet. Res., 43:289-293.

Madin, S.H., York, C.J., and McKercher, D.G., 1956. Infectious bovine rhinotracheitis virus. Science, 124:721-722.

Markwell, M.K., 1982. A new solid-state reagent to iodinate proteins I. Conditions for the efficient labeling of antiserum. Anal. Biochem., 125:427-432.

Marshall, R.L., Rodriguez, L.L., and Letchworth III., 1986. Characterization of envelope proteins of infectious bovine rhinotracheitis (Bovine Herpesvirus 1) by biochemical and immunological mehthods. J. Virol., 57:745-753.

Matthews, R.E.F., 1982. Classification and nomenclature of viruses. 4th Report Int. Comm. Tax. Viruses. Intervirol., 17:1-199.

McKercher, D.G., 1959. Infectious bovine rhinotracheitis. Adv. Vet. Sci. Comp. Med., 5:299-328.

McKercher, D.G., 1973. Viruses of other vertebrates. In: The herpesviruses. Kaplan, A.S.,(ed), Acad. Press, N.Y., p. 247-493.

McKercher, D.G., and Crenshaw, G.L., 1971. Comparative efficacity of intranasally and parentrally administered infectious bovine rhinotracheitis vaccines. JAVMA, 259:1362-1369.

McKercher, D.G., and Wada, E.M., 1964. The virus of infectious bovine rhinotracheitis as a cause of abortion in cattle. JAVMA, 144:136-142.

McNamara, M.K., Ward, R.E., and Kohler, H., 1985. Monoclonal idiotype vaccine against Streptococcus pneumoniae infection. Science, 226:1325-1326.

Mechor, G.D., Rousseaux, C.G., Radostits, O.M., Babiuk, L.A., and Petrie, L., 1987. Protection of newborn calves against fatal multisystemic infectious bovine rhinotracheitis by feeding colostrum from vaccinated cows. Can. J. Vet. Res., 51:452-459.

Melnick, J.L, 1985. Virus vaccines: An overview. In: High technology route to virus vaccines. Poc. Ann. Southwest Found. Biomed. Res., Int. Symp., Dreesman, G.R., Bronson, J.G., and Kennedy, R.C., (eds), Am. Soc. Microbiol., Washington, D.C., p. 1.

Metzler, A.E., Matile, H., Grassmann, U., Engels, M., and Wyler, R., 1985. European isolates of bovine herpesvirus-1: A comparison of restriction endonuclease sites, polypetides, and reactivity with monoclonal antibodies. Arch. Virol., 85:57-69.

Metzler, A.E., Schudel, A.A., and Engels, M., 1986. Bovine herpesvirus-1: Molecular and antigenic characteristics of variant viruses isolated from calves with neurological disease. Arch. Virol., 87:205-217.

Meyers, J.D., Fluornoy, N., and Thomas, E.D., 1980. Infection with herpes simplex virus and cell-mediated immunity after marrow transplant. J. Infec. Dis., 142:338-346.

Misra, V., Blumenthal, R.M., and Babiuk, L.A., 1981. Proteins specified by bovine herpesvirus 1 (infectious bovine rhinotracheitis virus). J. Virol., 40:367-378.

Misra, V., Gilchrist, J.E., Weinmaster, G., Qaultiere, L., Van Den Hurk, S., and Babiuk, L.A., 1982. Herpesvirus-induced "early" glycoprotein: Characterization and possible role in immune cytolysis. J. Virol., 43:1046-1054.

Mohanty, S.B., Lillie, M.G., Corselius, N.P., and Beck, J.D., 1972. Natural infection of IBR virus in goats. JAVMA, 160:879-880.

Molinaro, C.A., and NaKamura, R.M., 1982. Production and clinical applications of monoclonal antibodies in the clinical laboratory. NaKamura, R.M., Dita, W.R., and Tucker, E.S., (eds), Immunol. Anal. Masson Pub., p. 129-143.

Moss, B., 1985. Genetic engineering of live recombinant vaccinia virus vaccines. In: High technology route to virus vaccines. Pro. Ann. Southwest Found. Biomed. Res., Dreesman, G.R., Bronson, J.G., and Kennedy, R.C., (eds), p.69-74.

Msolla, P.M., 1979. Vaccination against infectious bovine rhinotracheitis virus. Vet. Rec., 104:535.

Murphy, B.R., and Chanock, R.M., 1985. Immunization against viruses. In: Fields Virology. Fields, B.N., (ed), Raven Press, N.Y., p. 349-370.

Narita, M., Inui, S., Namba, K., and Shimizu, Y., 1976. Trigeminal ganglionitis and encephalitis in calves intranasally inoculated with infectious bovine rhinotracheitis virus. J. Comp. Pathol., 86:93-100.

Nettleton, P.F., and Sharp, J.M., 1980. Infectious bovine rhinotracheitis virus excretion after vaccination. Vet. Rec., 107:379.

Nettleton, P.F., Sharp, J.M., Herring, A.J., and Herring, J.A., 1984. Infectious bovine rhinotracheitis virus excretion after vaccination, challenge, and immunosuppression. In: Latent herpesvirus infections in veterinary medicine. Wittmann, G., Gaskell, R.M., and Rziha, H.-J., (eds), Martinus Nijhoff Publishers, The Hague, p. 191-209.

Nisonoff, A., and Lamoyi, E., 1981. Implications of the presence of an internal image of the antigen in anti-idiotypic antibodies: Possible application to vaccine production. Clin. Immunol. Immunopathol., 21:397-406.

Nossal, G.J.V., and Lederberg, J., 1958. Antibody production by single cells. Nature, 181:1419-1420.

Okada, Y., 1962. Analysis of giant polynuclear cell formation caused by HVJ virus from Ehrlich's ascites tumour cells. I. Microscopic observation of giant polynuclear cell formation. Exp. Cell Res., 26:98-107.

Okazaki, K., Honda, E., Minetoma, T., and Tetsue, K., 1986. Mechanisms of neutralization by monoclonal antibodies to different antigenic sites on the bovine herpesvirus type 1 glycoproteins. Virol., 150:260-264.

Ollier, P., Rocca-Serra, J., Somme, G., Theze, J., and Fougereau, M., 1985. The idiotypic network and the internal image possible regulation of a germ-linked network by paucigene encoded Ab2 (anti-idiotypic antibodies) in the GAT system. EMBO J., 4:3681-3688.

Oudin, J., and Michel, M., 1963. Une novelle forme d'allotypie des globulines du serum de lapin apparemment lie'e a' la fonction et a' la specificite anticorps. C. R. Seances Acad. Sci., 257:805-808.

Panicali, D., and Paoletti, E., 1982. Construction of poxviruses as cloning vectors: Insertion of thymidine kinase gene of herpes simplex virus into the DNA of infectious vaccinia virus. Proc. Natl. Acad. Sci., 79:4927-4931.

Paoletti, E., Lipinskas, B.R., Samsonoff, C., Mercer, S., and Panicali, D., 1984. Construction of the live vaccines using genetically engineered poxviruses: Biological activity of vaccinia virus recombinants expressing the HBV surface antigen and herpes simplex virus glycoprotein D. Proc. Natl. Acad. Sci., 81:193-197.

Pastoret, P.-P., Aguilar-Setien, A., Burtonboy, G., Mager, J., Jetteur, P., and Schoenaers, F., 1979. Effect of repeated treatment with dexamethasone on the re-excretion pattern of infectious bovine rhinotracheitis virus and humoral immune response. Vet. Microbiol., 4:149-155.

Pastoret, P.-P., Babiuk, L.A., Misra, V., and Griebel, P., 1980. Reactivation of temperature-sensitive and non-temperature -sensitive infectious bovine rhinotracheitis vaccine virus with dexamethasone. Infec. Immunol., 29:483-488.

Pastoret, P.-P., Thiry, E., Brochier, B., and Derboven, G., 1982. Bovid herpesvirus-1 infection of cattle: Pathogenesis, latency, and consequences of latency. Ann. Rech. Vet., 13:221-235.

Pastoret, P.-P., Thiry, E., and Vindevoget, H., 1981. Problemes lies a la latence lors de la vaccination contre le virus de la rhinotracheite bovine infectieuse. Dev. Biol. Stand., 52:455-461.

Pospisil, Z., Krejci, J., and Rodak, C., 1983. Demonstration of colostral antibodies in the nasal secretion of calves and their protective effect against infection. Acta Vet. Brno., 52:59-65.

Potter, M., and Boyce, C.R., 1962. Induction of plasma cells neoplasms in strain Balb\c mice with mineral oil adjuvants. Nature, 193:1086-1087.

Reagan, K.J., 1985. Modulation of immunity to rabies virus induced by anti-idiotypic antibodies. Curr. Top. Microbiol. Immunol., 119:15-30.

Reagan, K.J., Wunner, W.H., Wiktor, T., and Kopowski, H., 1983. Anti-idiotypic antibodies induce neutralizing antibodies to rabies virus glycoprotein. J. Virol., 48:660-666.

Roberts, A.W., Carter, G.R., and Carter, F.A., 1974. Infectious bovine rhinotracheitis virus recovered from a milk of a cow with mastitis. JAVMA, 164:413.

Rock, D.L., Beam, S.L., and Mayfield, J.E., 1987. Mapping bovine herpesvirus type 1 latency-related RNA in trigeminal ganglia of latently infected rabbits. J. Virol., 61:3827-3831.

Rock, D.L., Hagemoser, W.A., Osorio, F.A., and Reed, D.E., 1986. Detection of bovine herpesvirus type 1 RNA in trigeminal ganglia of latently infected rabbits by in situ hybridization. J. Gen. Virol., 67:2515-2520.

Roizman, B., and Batterson, W., 1985. Herpesviruses and their replication. In: Fields Virology, Fields, B.N.,et al (eds), Raven Press, N.Y., p. 497-526.

Rosenquist, B.D., and Loan, R.W., 1969. Interferon production in the bovine species by infectious bovine rhinotracheitis virus. Am. J. Vet. Res., 30:1305.

Rossi, C.R., and Kiesel, G.K., 1982. Effect of infectious bovine rhinotracheitis virus on viral shedding in challenge-exposed calves treated with dexamethasone. Am. J. Vet. Res., 43:1576-1579.

Rouse, B.T., 1984a. Cell mediated immune mechanisms. In: Immunobiology of herpes simplex virus infection. Rouse, B.T., and Lopez, C., (eds), CRC Press, Boca Raton, FA., p. 107-120.

Rouse, B.T., 1984b. Role of adaptive immune mechanisms in herpes simplex resistance. In: Immunobiology of herpes simplex virus infection. Rouse, B.T., and Lopez, C., (eds), CRC Press, Boca Raton, FA., p. 131-144.

Rouse, B.T., 1985. Immunobiology of herpesvirus infections. In: Immunity to herpesvirus infections of domestic animals. Pastoret, P.-P., Thiry, E., and Saliki, J., (eds), CEC, Brussels, p. 11-24.

Rouse, B.T., and Babiuk, L.A., 1975. Host defense mechanism against IBR virus. II. Inhibition of viral plaque formation by immune peripheral lymphocytes. Cell Immunol., 17:43-56.

Rouse, B.T., and Babiuk, L.A., 1977. The direct antiviral cytotoxicity by bovine lymphocytes is not restricted by genetic incompatibility of lymphocytes and target cells. J. Immunol., 118:618-624.

Rouse, B.T., Grewal, A.S., Babiuk,L.A., and Fujimiya, Y., 1977. Enhancement of antibody-dependent cell-mediated cytotoxicity of herpesvirus-infected cells by complement. Infect. Immunol., 18:660-665.

Rouse, B.T., and Horohov, D.W., 1984. Cytotoxic T lymphocytes in herpesvirus infections. Vet. Immunol. Immunopathol., 6:35-66.

Rouse, B.T., Wardley, R.C., and Babiuk, L.A., 1976. Antibody-dependent cell-mediated cytotoxicity in cows: Comparison of effector cell activity against heterologous erythrocytes and herpesvirus-infected bovine target cells. Infect. Immunol., 13:1433-1439.

Rubinstein, L.J., Goldberg, B., Hiernaux, J., Stein, K.E., and Bona, C.A., 1983. Idiotype-antiidiotype regulation. V. The requirement for immunization with antigen or monoclonal anti-idiotypic antibodies for the activation of B2-6 and B2-1 polyfructosan reactive clones in Balb/c mice treated at birth with minute amounts of anti-A48 idiotype antibodies. J. Exp. Med., 158:1129-1144.

Sacks, D.L, Esser, K., and Sher, A., 1982. Immunization of mice against African trypanosomiasis using anti-idiotypic antibodies. J. Exp. Med., 115:1108-1119.

Saxegaard, F., and Ontad, O., 1967. Isolation and identification of IBR-IPV virus from cases of vaginitis and balanitis in swine and from healthy swine. Nord. Vet. Med., 19:54-57.

Schudel, A.A., Carrillo, B.J., Wyler, R., and Metzler, A.E., 1986. Infection of calves with antigenic variants of bovine herpesvirus 1 (BHV-1) and neurological disease. J. Vet. Med., 33:303-310.

Schwartz, A.J.F., York, C.J., Zirbel, L.W., and Estella, L.A., 1957. Modification of infectious bovine rhinotracheitis (IBR) virus in tissue culture and development of a vaccine. Proc. Soc. Exp. Biol. Med., 96:453-458.

Schwartz, A.J.F., Zirbel, L.W., Estella, L.A., et al, 1958. Propagation and modification of infectious bovine rhinotracheitis (IBR) virus in porcine kidney tissue culture. Proc. Soc. Exp. Biol. Med., 97:680-683.

Seal, B.S., St. Jeor, S-C., and Taylor, R.E., 1985. Restriction endonuclease analysis of bovine herpesvirus 1 DNA and nucleic acid homology between isolates. J. Gen. Virol., 66:2787-2792.

Sege, K., and Paterson, P.A., 1978a. Use of anti-idiotypic antibodies as cell surface receptor probes. Proc. Natl. Acad. Sci., 75:2443-2447.

Sege, K., and Paterson, P.A., 1978b. Anti-idiotypic antibodies against anti-vitamin A transporting protein react with prealbumin. Nature, 271:167-168.

Sharpe, A.H., Gaulton, G.N., McDade, K.K., Fields, B.N., and Greene, M.I., 1984. Syngeneic monoclonal anti-idiotype can induce cellular immunity to reovirus. J. Exp. Med., 160:1195-1205.

Sheffy, B.E., and Davies, D.H., 1972. Reactivation of bovine herpesvirus after corticosteroid treatment. Proc. Soc. Exp. Biol. Med., 140:947-976.

Sherr, D.H., Ju, S.T., and Dorf, M.E., 1981. Hapten-specific T-cell responses to 4-hydroxy-3-nitrophenyl acetyl. XII. Fine specificity of anti-idiotypic suppressor T-cells (Ts2). J. Exp. Med., 154:1382-1389.

Smith, P.C., 1976. The bovine herpesvirus: An overview. In: Proc. 80th Ann. Meeting, U.S. Anim. Health Assoc., 149:147.

Snowdon, W.A., 1965. The IBR-IPV viruses: Reaction to infection and intermittant recovery of virus from experimentally infected cattle. Aust. Vet. J., 135-142.

Splitter, G.A., Eskra, L., Miller-Edge, M., and Splitter, J.L., 1985. Bovine herpesvirus 1: Interactions between animal and virus. In: Comparative pathology of viral diseases. vol. 1., Olson, R.G., et al, (eds), CRC Press, Boca Raton, FA., p. 57-88.

Staerz, U.D., Rammensee, H.G., Benedetto, J.D., and Bevan, M., 1985. Characterization of a murine monoclonal antibody specific for an allotypic determinant on the T-cell antigen receptor. J. Immunol., 134:3994-4000.

Stauber, E.H., Autenrieth, R., Markham, O.D., and Whitbeck, V., 1980. A seroepidemiologic survey of three pronghorn (Antilocapra americana) populations in southeastern Idaho, 1975-1977. J. Wildlife, 16:109-115.

Stein, K., and Soderstrom, T., 1984. Neonatal administration of idiotype or anti-idiotype primes for protection against E. coli K13 infection in mice. J. Exp. Med., 160:1001-1011.

Straub, O.C., 1976. Prophylactic measures against infectious bovine rhinotracheitis. Deutsch Tierarztliche Wochenschrift, 83 (6):270.

Straub, O.C., 1977. Die Sanierung einer Besammungstation von IBR-IPV virus ausscheidern durch einen Einsatz von IBR-IPV lebeud Impsloff. Dtsch. Tieraerztl Wochenschr, 84:259-264.

Strokes, C.R., 1985. Local immunity to herpesviruses. In: Immunity to herpesvirus infections of domestic animals. Pastoret, P.-P., Thiry, E., and Saliki, J., (eds), CEC, Brussels, p. 51-57.

Tanaka, M., Sasaki, N., and Seto, A., 1986. Induction of antibodies against newcastle disease virus with syngeneic anti-idiotype antibodies in mice. Microbio. Immunol., 30:323-331.

Todd, J.D., Volence, F.J., and Paterson, I.M., 1971. Intranasal vaccination against IBR: Studies on early onset of protection and use of the vaccine in pregnant cows. JAVMA, 159:1370-1374.

Tousimis, A.J., Howells, W.V., et al, 1958. Biophysical characterization of infectious bovine rhinotracheitis virus. Proc. Sci. Exp. Biol. Med., 99:614-617.

Trepanier, P., Minocha, H.C., Bastein, Y., et al, 1986. Bovine herpesvirus 1: Strain comparison of polypeptides and identification of a neutralization epitope on the 90-kilodalton hemagglutinin. J. Virol., 60:302-306.

Urbain, J., 1986. Idiotypic network: A noisy background or a breakthrough in immunological thinking. The broken mirror hypothesis. Ann. Inst. Pasteur Immunol., 137C:57-64.

Urbain, J., Wikler, M., Franssen, J.D., and Collignon, C., 1977. Idiotypic regulation of the immune system by the induction of antibodies against anti-idiotypic antibodies. Proc. Natl. Acad. Sci., 74:5126-5130.

Uytdehaag, F.G.C., and Osterhaus, A.D.M.E., 1985. Induction of neutralizing antibody in mice against poliovirus type II with monoclonal anti-idiotypic antibody. J. Immunol., 134:1225-1229.

Van Drunen Littel-van den Hurk, S., and Babiuk, L.A., 1985. Antigenic and immunogenic characteristics of bovine herpesvirus type 1 glycoproteins GVP 3/9 and GVP 6/11a/16, purified by immunoadsorbent chromatography. Virol., 144:204-215.

Van Drunen Littel-van den Hurk, S., Van Den Hurk, J.V., and Babiuk, L.A., 1985. Topographical analysis of bovine herpesvirus 1 glycoproteins: Use of monoclonal antibodies to identify and characterize fuctional epitopes. Virol., 144:216-227.

Van Drunen Littel-van den Hurk, S., Van Den Hurk, J.V., Gilchrist, J.E., Misra, V., and Babiuk, L.A., 1984. Interactions of monoclonal antibodies and bovine herpesvirus type 1 (BHV-1) glycoproteins: Characterization of their biochemical and immunological properties. Virol., 135:466-479.

Victor-Korbin, 1988. Regulatory function of anti-idiotype autoantibodies. In: Biological application of anti-idiotypes. vol. 1. Bona, C.A., (ed), CRC Press, Boca Raton, FA., p. 105-132.

Watrach, A.M., and Bahnemann, H., 1966. The structure of infectious bovine rhinotracheitis virus. Arch. Ges. Virusforsch., 18:1-7.

Watson, R.J., Weis, J.H., Salstrom, J.S., and Enquist, L.W., 1982. Herpes simplex virus type-1 glycoprotien D gene: Nucleotide sequence and expression in E.coli. Science, 218:381-384.

Wellemans, G., and Lenmen, J., 1973. La rhinotracheite infectieuse des bovins (IBR) et sa serologie. Ann. Med. Vet., 117:507-518.

Whitman, J.E., and Hetrick, F.M., 1965. An indirect HA test for detecting antibody to infectious bovine rhinotracheitis virus. Cornell Vet., 55:613-622.

Wilkie, B.N., 1982. Respiratory tract immune reponse to microbial pathogens. JAVMA, 181:1074-1079.

Wilson, T.E., 1974. Observations and comments on two outbreaks of abortion associated with IBR viruses. Can. Vet. J., 15:227-229.

Yap, K.L., Ada, G.L., and McKenzie, I.F., 1978. Transfer of specific cytotoxic T lymphocytes protects mice inoculated with infleunza virus. Nature, 273:238-240.

Yelverton, E., Norton, S., Obijes, J.F., and Goeddel, D.V., 1983. Rabies virus glycoprotein analogues: Biosynthesis in E.coli. Science, 219:614-620.

York, C.J., Schwartz, A.J.F., and Estella, L.A., 1957. Isolation and identification of infectious bovine rhinotracheitis virus in tissue culture. Proc. Soc. Exp. Biol., 94:740-744.

Zarnke, R.L., and Yuill, T.M., 1981. Serologic survey for selected microbial agents in mammals from Albrta, 1976. J. Wildlife Dis., 17:453-461.

Zuscheck, R., and Chow, T.L., 1961. Immunogenicity of two infectious bovine rhinotracheitis vaccines. JAVMA, 139:236-237.

# COMPARISON OF RABBIT POLYCLONAL ANTI-IDIOTYPES TO BOVINE HERPESVIRUS-1

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# ABSTRACT

Mouse monoclonal antibodies (Mabs) to bovine herpesvirus-1 (BHV-1) were produced and characterized. Balb\c mice (8 to 9 weeks old) were immunized with purified BHV-1, boosted, and sacrificed for fusion. Splenocytes were fused with mouse myeloma cells P3/X63/Ag8.653 and the resultant hybridomas were selectively grown in hypoxanthine-aminopterin-thymidine medium. Screening of hybridomas by ELISA revealed 25 BHV-1 specific clones, of them 12 stable Mab producing cell lines were fully characterized. Antigen specificities of the Mabs were determined by immunoprecipitation of $S_{35}$-methionine-labeled BHV-1 polypeptides. One group of Mabs reacted with glycoproteins (gps) 130Kd\75Kd, and 50Kd, a second group recognized gp 77Kd, and a third group precipitated gp 97Kd. Four Mabs were selected from this panel to produce anti-idiotypic antibodies (anti-id) in rabbits. Three of the Mabs possessed neutralizing activity and reacted against the 130\75\50Kd, 77Kd, and 97Kd gps respectively. The fourth non-neutralizing Mab reacted against the 97Kd gp. All four Mabs recognized different epitopes on the virion as determined by compitition radioimmunoassay. Mabs ascites fluids were obtained , and purified on protein A-sepharose affinity column.

Female New Zealand White rabbits were immunized with 300 ug of purified Mab. Three doses were injected at biweekly intervals. Antisera were collected periodically throughout

the immunization schedule and analysed for anti-id by ELISA. The highest idiotype-specific activity was detected in serum samples collected two weeks after the initial injection. Pooled rabbit sera of different samples at this period were purified on affinity column made by coupling normal mouse IgG to affi-gel 10, to remove anti-isotypic and anti-allotypic antibodies. The flowthrough from the column was again passed over protein A-sepharose column to isolate idiotype-specific IgGs.

The purified anti-id were characterized by competitive ELISA. The binding of BHV-1 Mabs to BHV-1 was inhibited by the respective anti-id, suggesting that, the anti-id were specific to the variable region of the antigen combining site of Mab and probably mimic the epitope structure on the virus. In order to determine the presence or absence of cross-reactive idiotopes among the Mabs used for the production of anti-id, each purified anti-id preparation was tested against homologous and heterologous Mab by ELISA. Each anti-id was specific, with a high titer, to its corresponding Mab suggesting absence of cross-reactive idiotopes.